THE EFFECTS OF HYBRIDIZATION ON SKELETAL MORPHOLOGY IN TWO CLOSELY RELATED POPULATIONS OF RHESUS MACAQUES (*MACACA MULATTA*): A GEOMETRIC MORPHOMETRIC APPROACH

by

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ABSTRACT

THE EFFECTS OF HYBRIDIZATION ON SKELETAL MORPHOLOGY IN TWO CLOSELY RELATED POPULATIONS OF RHESUS MACAQUES (*MACACA MULATTA*): A GEOMETRIC MORPHOMETRIC APPROACH

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Precise identification and classification techniques are vital for the field of paleoanthropology to ensure that hominin fossilized remains are labeled accurately. The morphology of extinct hominin specimens will typically be compared to extant nonhuman primate species because of how closely related they are phylogenetically. Observable similarities in their morphological variation can be examined to infer which traits may be a result of evolution and this can update our understanding of their evolutionary relationships. The genus *Macaca* displays a level of morphological variation that is similar to that seen in the genus *Homo*, therefore macaques can be used as an analogous model to study morphological variation as it relates to hominin species. Geometric morphometrics is an approach that can quantitatively analyze the morphology of a specimen to infer questions relating to accurate identification and classification techniques. Recently, research has begun implementing a geometric morphometric approach to examine the morphological variation between- and within-species of macaques to develop a comparative dataset that can used with other closely related primate species, like hominins. Here, a sample of hybrid rhesus macaques (*M. mulatta*) are used to investigate the effects of hybridization on skeletal morphology and to expand the existing comparative dataset to offer a more inclusive model for hominin species. The skull and os coxa

from the hybrid *M. mulatta* sample are quantitively examined via a geometric morphometric approach to determine whether specimens could be allocated to their correct taxonomic identification at the individual- and group-levels. In addition, the skull and os coxa are compared to determine which bone can more correctly allocate specimens. The goal of the thesis is to determine whether this quantitative approach can be used on a hybrid nonhuman primate sample as seen in previous research on purebred hominin and nonhuman primate samples. Results from the statistical analyses indicated that the hybrid *M. mulatta* were unable to be clearly differentiated based on their morphological variation. The results indicate that at this level of speciation, the quantitative approach is unable to differentiate specimens regardless of whether they are a purebred or hybrid individual. As a whole, the results from this thesis support the idea that there is a complex interaction between hybridization and morphology that has only started to be addressed in the literature. Future research is warranted to further examine the morphological variation of hybrid hominin and nonhuman primate species, which will ultimately update our understanding of evolutionary history.

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Chapter 1: Introduction

Paleoanthropology is the interdisciplinary branch of biological anthropology that studies the origins and development of hominins from an evolutionary standpoint. A substantial body of research has been devoted to examining hominin fossil forms and the significance of their traits. Current research (e.g., Green et al., 2010; Kuhlwilm et al., 2016; Schroeder and Ackermann, 2017; Slon et al., 2018; Ackermann et al., 2019) focuses on investigating when, where, and how hominins emerged from their hominoid ancestors, and elucidating how anatomically modern *Homo sapiens* interacted with other contemporary hominin taxa, such as Neanderthals and Denisovans, in the past. To address these research topics, paleoanthropologists must accurately identify and classify the fossilized remains believed to belong to hominin species to better understand their evolutionary history (Tattersall, 1986; Aiello et al., 2000; Baab 2008; von Cramon-Taubadel and Smith, 2012; Smith and von Cramon-Taubadel, 2015; Fabre et al., 2018). Through comparing one specimen to another, the researcher can answer questions relating to their taxonomy and phylogeny (Nunn, 2011). That said, there are a multitude of factors that can complicate the accurate identification of the hominin fossil record, including the limited and often incomplete nature of specimens recovered, individual biases introduced on behalf of the researchers, and the presence of anatomical features that closely resemble other species in the genus (Tattersall, 1992; Wood and Boyle, 2016; Kenyon-Flatt, 2020).

In recent decades, paleoanthropologists have recognized these limitations and suggested the implementation of a comparative framework to accurately understand the

evolutionary history of our own species and our ancestral hominin species (Ackermann and Bishop, 2009; von Cramon-Taubadel and Lycett, 2014; Zollikofer et al., 2014; Roseman and Auerbach, 2015; Smith and von Cramon-Taubadel, 2015; Ackermann et al., 2019; Kenyon-Flatt, 2020). Given that anatomically modern *H. sapiens* is the only extant species in the genus *Homo*, it is imperative to compare hominin fossilized remains to other extant analogous species (Aiello et al., 2000; Ravosa and Profant, 2000; Ackermann, 2003; Baab, 2008; von Cramon-Taubadel and Smith, 2012; Zollikofer et al., 2014; Roseman and Auerbach, 2015; Smith and von Cramon-Taubadel, 2015; Kenyon-Flatt, 2020). When conducting research on evolutionary history, nonhuman primates are a useful comparative model for hominins. Paleoanthropologists can study the morphology of nonhuman primates to infer how the observable morphological variation present in these species could compare to that of extinct hominin species. Given that hominins and nonhuman primates are closely related phylogenetically, any observable similarities in morphology could be a byproduct of evolution and can update our understanding of their evolutionary relationships (von Cramon-Taubadel and Lycett, 2014; Zollikofer et al., 2014; Monson et al., 2017; Kenyon-Flatt, 2020).

The order Primates is one of the most diverse orders of mammals and is estimated to comprise over 500 species, including anatomically modern *H. sapiens* (Wu et al., 2022). The obvious choice when trying to create a comparative framework to study hominin fossilized remains would be to select the most closely related extant nonhuman primate species (see Fig. 1). These species would be morphologically similar to hominins, which would aid in comparing similarities or differences in their skeletal morphology as it relates to evolution. Belonging to the same subfamily Homininae, the genus *Pan* is the most recent to share a common ancestor

with hominins (Fig. 1). Unfortunately, *Pan,* which includes common chimpanzees and bonobos, is not a model taxon to compare to hominins in the context of morphological variation. Anatomically modern *H. sapiens* are distributed globally and, as a result, have adapted to a multitude of diverse habitats (Kenyon-Flatt, 2020). Additionally, fossil evidence has suggested that there may have been as many as thirty-one distinct hominin species discovered in the field thus far (Wood and Boyle, 2016). *Pan* is only found in Africa and there are currently five recognized species (ITIS, 2022), making them a weak model for examining the diverse morphological variation that exists in the genus *Homo.* Moving further away from hominins, the next group of most closely related extant primate taxa to consider are *Gorilla* (gorillas) and *Pongo* (orangutans), the other great apes (Fig. 1). Similar to *Pan,* gorillas and orangutans are each only native to one continent, Africa and Asia, respectively, and have a combined total of five recognized species (ITIS, 2022). Taken together, the most closely related extant species to the genus *Homo* offer a poor comparative model in terms of geographic and taxonomic variation to adequately examine the morphological diversity of the hominin fossil record (Kenyon-Flatt, 2020).

Researchers have therefore turned to more distantly related nonhuman primate taxa that could still offer the unique opportunity to measure morphological diversity and serve as an analog to hominin species. Macaques are known as a taxonomically rich genus of Cercopithecine monkey that have received much attention by researchers because of their uniqueness within the order Primates. The genus *Macaca* is recognized as having the most successful nonhuman primate radiation, behind the genus *Homo*, and has a wide geographic range that includes Africa, Asia, Europe, and the Middle East (Fa, 1989; Hayasaka et al., 1996;

Morales and Melnick, 1998; Deinard and Smith, 2001; Abegg and Thierry, 2002; Tosi et al., 2003; Weinstein, 2011; Zinner et al., 2011; Ito et al., 2014; Roos and Zinner, 2015; Jiang et al., 2016; Grunstra et al. 2018; Roos et al., 2019). Throughout their evolutionary history, macaques have adapted to a multitude of habitats, and, as a result, there is a significant amount of variation observed in the twenty-three recognized extant species (Kenyon-Flatt, 2020; Kenyon-Flatt et al., 2020; ITIS, 2022).

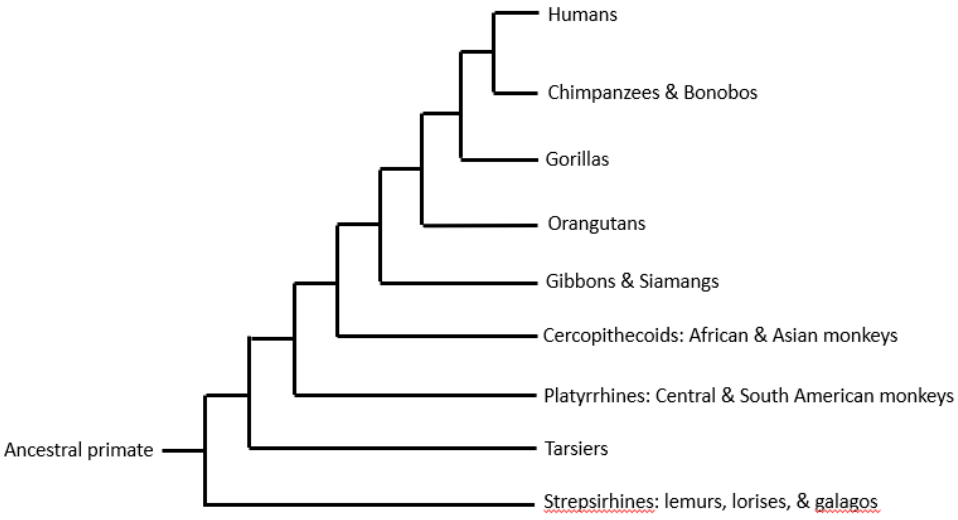


Figure 1: Primate phylogenetic tree.

In addition, fossil and genetic research agree that the genus *Macaca* originated within Africa and later migrated to the multitude of habitats occupied by its species today (Fa, 1989; Roos et al., 2019). Due to the exceedingly large levels of morphological variation in the genus, the phylogenetic and taxonomic classification of macaques has caused much debate in the literature (see Fa, 1989; Morales and Melnick, 1998; Deinard and Smith, 2001; Evans et al., 2003; Weinstein 2011; Liedigk et al., 2014; Jiang et al., 2016; Roos et al, 2019, Kenyon-Flatt et al., 2020). Macaques are therefore a prime candidate to study comparative morphological variation because of their similarities with hominins, with a growing body of research

recognizing the importance of macaque morphological variation as it provides insights into overall primate evolution and the interpretation of the hominin fossil record (e.g., Jolly, 2001; Weinstein, 2011; Smith and von Cramon-Taubadel, 2015; Jiang et al., 2016; Monson et al., 2017).

Identifying a model nonhuman primate species that can be used as a tool for comparison to hominins is only the first step when undertaking research of this nature. Next, it is necessary to determine which method would be most accurately able to compare the morphological variation of genus *Macaca*. Traditionally, it was standard to take newly recovered nonhuman primate or hominin fossilized remains and compare them to existing fossils, for which the taxonomic classification was already labeled, thereby allowing them to serve as a tool for comparison. This method of classification was qualitative in nature, which involved researchers utilizing their own observational skills to identify similarities in the morphology of skeletal elements (Aiello et al., 2000; Ackermann, 2003; Zollikofer et al., 2014; Monson et al., 2017; Kenyon-Flatt, 2020). In cases where unknown nonhuman primate or hominin fossilized remains were concerned, researchers had to use any existing available skeletal elements to try and accurately identify the specimen(s). As more assemblages were discovered, paleoanthropologists from different backgrounds began to identify similarities in the observed morphological variables based on their own interpretations. Debate arose when researchers started to identify nonhuman primate or hominin fossilized remains differently, thus introducing research biases and indicating that a new classification method was necessary (Kenyon-Flatt, 2020).

Another qualitative and observational trend that was historically common in the field of paleoanthropology was favoring the cranium and its associated skeletal elements when making an identification of unknown specimens. When prioritizing the skull over other available skeletal elements, researchers were convinced that it was straightforward to determine if a specimen was a nonhuman primate or a human based on the visual differences between skulls. As a result, postcranial skeletal elements were often overlooked or ignored completely when a skull was present (Ravosa and Profant, 2000; Roseman, 2004; Fleagle et al., 2010; von Cramon-Taubadel and Lycett, 2014; Smith and von Cramon-Taubadel, 2015; Fleagle et al., 2016). This pattern among researchers became problematic with newly discovered assemblages of hominins when postcranial skeletal elements displayed more morphological variation than the cranial skeletal elements (Manzi et al., 2001; Gilbert et al., 2003; White, 2003). More specifically, assessing taxonomic identification based solely on the skull proved difficult with assemblages *of Homo habilis* (Kramer et al., 1995), *Homo floresiensis* (Morwood et al., 2005), and *Homo erectus* (Turner and Chamberlain, 1989; Antón, 2002; Asfaw et al., 2002; Manzi et al., 2003; Suwa et al., 2007; Terhune et al., 2007; Zollikofer et al., 2014). These examples suggested that accurate taxonomic identifications and/or classifications would not be as accurate if the cranial skeletal elements were the main consideration when examining morphology. Furthermore, there were many instances where limited skeletal elements were recovered and/or a cranium was absent (e.g., Walker et al., 2011; Harcourt-Smith et al., 2015; Villmoare et al., 2015; Feuerriegel et al., 2016). Although many researchers still prioritize the skull when identifying unknown fossilized remains, there has been considerable progress in recognizing the necessity to view all available skeletal elements equally (e.g., Young, 2008; Lycett and von

Cramon-Taubadel, 2013; von Cramon-Taubadel and Lycett, 2014; Kenyon-Flatt, 2020; Kenyon-Flatt et al., 2020). In conclusion, these previous trends that utilized qualitative methods to identify unknown nonhuman primate or hominin fossilized remains are challenging and can lead to imprecise classifications in the field.

Accordingly, a growing number of projects began implementing quantitative methods to examine the morphological variation of both known and unknown nonhuman primate or hominin fossilized remains. For many years, researchers collected and compared linear measurements of specimens. However, the development of geometric morphometric techniques allowed researchers to quantify form in a novel way. Geometric morphometrics (GM), which labels landmarks on skeletal elements to analyze variables specifically related to morphology via statistical, rather than observational, methods (MacLeod, 2002; Richtsmeier et al., 2002; Slice, 2007; von Cramon-Taubadel et al., 2007; Klingenberg, 2010; Cooke and Terhune, 2015), became popular in the field of biological anthropology, and more specifically in paleoanthropology, when researchers began asking questions about shape differences among organisms. This specific technique permits researchers to obtain metric data that can quantify specific morphological traits and address research questions pertaining to taxonomy and/or phylogeny, in addition to those surrounding the identification and classification of a specimen (MacLeod, 2002; Richtsmeier et al., 2002; Slice, 2007; Cooke and Terhune, 2015; Kenyon-Flatt, 2020). GM methods can address variation in 2-dimensional or 3-dimensional shape while preserving the integrity of the specimen's form. Regardless of which dimension the techniques are observed in, differences in shape and size can be examined to infer their relationship (MacLeod, 2002; Slice, 2007).

Initially, the main goal of studies that utilized a GM approach was to analyze the degree of morphological variation that was observed in a single skeletal element resulting from external factors. For example, the cranial morphology of large bodied African monkeys (Frost et al., 2003), various populations of *Homo erectus* (Terhune, 2007; Baab, 2008; Baab, 2010), and multiple species of macaques (Ito et al., 2014) has been examined to determine how varying environmental and behavioral factors could have contributed to these species' morphological variation. In addition, the distal limb morphology of Neanderthals (Roses et al., 2017) and forelimb morphology of various strepsirhines (Fabre et al., 2018) have been investigated for similar reasons. Taken together, these studies provide evidence for the use of this quantitative method when examining the morphology of specific skeletal elements from individual nonhuman primate or hominin species.

Additional studies have used GM methods on skeletal elements with the intention of comparing different genera of nonhuman primates or hominins. For instance, skeletal morphology has been comparatively analyzed via GM techniques on their cranial morphology (e.g., Lockwood et al., 2002; Smith, 2009; Fleagle et al., 2010; Fleagle et al., 2016), scapulae (Young, 2008; Green et al., 2016), and pelvic morphology (Lycett and von Cramon-Taubadel, 2013). This comparative component of GM allowed these researchers to examine the observed morphological variation present in multiple species and genera to help answer questions regarding their taxonomy and phylogeny. As a result, current assumptions about their evolutionary relationships could be supported, or updated, among both nonhuman primates and hominins (Fleagle et al., 2010; Fleagle et al., 2016). It is important to highlight these studies that adapted GM methods to employ a comparative approach because there are implications

for how this method could be applied towards larger questions regarding classification in paleoanthropology.

In the context of known or unknown nonhuman primate and hominin fossils, GM methods can be used to recognize the morphological variation both between- and within-species of a genus to help make an accurate identification and classification. Given that the GM approach statistically measures morphological variables, this method could help recognize patterns in the morphology of newly recovered specimens or address those that have an identification that is contentious among researchers. More specifically, this comparative approach using GM techniques could be helpful when addressing taxa that are known for their exceedingly diverse morphological variation which make accurate identifications difficult, such as macaques and hominins. The genus *Macaca* has already been selected to serve as an analogous species in previous research, therefore it wouldn't be unreasonable to comparatively analyze this genus to help answer questions regarding the identification and classification of genus *Homo*. Consequently, the efficacy of utilizing GM methods to analyze the morphological variation in the genus *Macaca* is ongoing and shows considerable promise for how this method can improve the identification and classification processes.

Kenyon-Flatt et al. (2020) addressed the contentious classification of the genus *Macaca* and the ongoing attempts to improve the understanding of hominin evolution by implementing GM in a way that would specifically attempt to recognize and label macaque morphological variation. The researchers were interested in determining whether a quantitative method like GM could accurately identify and classify the members of closely related primate species like macaques based on their morphology. Two species from the genus were selected, *Macaca*

mulatta and *Macaca fascicularis,* and then another cercopithecoid monkey species, *Trachypithecus cristatus,* was added as an outgroup. The cranium and os coxa were scanned from each specimen due to the ongoing debate in the field on whether crania or postcrania were more reliable when making a correct classification. The main goals of this study were to determine if the morphology of each species was distinct enough for GM analyses to lead to correct taxonomic designations for specimens, while also measuring the accuracy of each skeletal element to know if one was more reliable than the other. Results from the study provided support for the use of GM methods when attempting to classify known or unknown macaque skeletal remains when specifically measuring the morphological variation of the cranium and os coxa. Furthermore, there were instances where the cranium and os coxa were both able to correctly identify specimens in the sample. The researchers concluded that the study provided enough evidence for undertaking future research of this nature. Additionally, they recognized including additional species from the genus *Macaca* or repeating the project with another closely related primate species would yield more information (Kenyon-Flatt et al., 2020).

In an effort to continue this research, Kenyon-Flatt (2020) expanded the study to include a larger sample from the genus *Macaca*. The main goal of this larger study was to determine how much morphological variation could exist within an exceedingly variable genus like *Macaca*, while also incorporating additional skeletal elements to represent a larger proportion of the skeleton. The ultimate goal was to create a comparative dataset starting with macaques that could be used for hominin species given the morphological similarities between genus *Macaca* and *Homo*. Eight species of macaques were selected to represent each of the currently

recognized species-groups in genus *Macaca,* and *T. cristatus* was again included as an outgroup. Morphological variation was analyzed among eight skeletal elements via GM data including the cranium, mandible, scapula, humerus, radius, os coxa, femur, and tibia, with the goal to determine which areas of the skeleton provided reliable morphological signals to make an accurate taxonomic classification. In addition, the effects of climate, geography, locomotor behaviors, and dietary preferences were considered to establish whether these external factors had any influences on the accuracy of the analyses (Kenyon-Flatt, 2020).

The results from this study were informative for multiple reasons. First, the study was largely successful in that the identity of the specimens was not continuously confused among species regardless of which skeletal element was in question. Second, the skeletal elements belonging to the postcrania were just as successful, and in some instances more so, at accurately labeling the specimen back to its correct species. Third, a comparative dataset of macaque morphological variation was created that included additional skeletal elements to better represent the entire skeleton. Fourth, the results provided support for how the research could potentially be mirrored with a hominin sample. Lastly, the results indicated that ecogeographic variables were shown to have an impact on the morphological variation observed among the eight species of macaques, whereas the locomotor and behavioral variables did not. However, these impacts did not influence the accuracy of GM methods in determining the identity of the specimens (Kenyon-Flatt, 2020). Taken together, these results provide ample support for the use of this quantitative method over previous qualitative methods when examining known or unknown skeletal remains. Furthermore, a baseline was

created to aid future research that is interested in asking questions regarding the taxonomic and/or phylogenetic classification of primates and hominins.

Here I focus on addressing the accuracy of using a geometric morphometric approach to correctly identify and classify macaque specimens that are of hybrid origin. More specifically, I seek to strengthen the research that has developed a comparative dataset of macaque morphological variation through the inclusion of hybrid macaques comprised of two regional variants of *M. mulatta*. It is the goal of the current project to develop a more inclusive model for which hominin specimens could be accurately identified and quantitatively classified. To date, the research that focuses on nonhuman primate or hominin specimens has only utilized GM methods on purebred representative species. Given that the genus *Macaca* has an abundance of species living in multiple diverse habitats, it must also be noted that there are examples of neighboring macaque populations interbreeding to produce fertile hybrid offspring (Ackermann et al., 2006; Ackermann and Bishop, 2009). Examples of macaque hybridization have been documented between populations of *M. fascicularis* and *M. mulatta* (Malaivijitnond et al., 2008; Bonhomme et al., 2009; Yao et al., 2017), *M. fascicularis* and *M. sinica* (Zinner et al., 2011), *M. fascicularis* and *M. nemestrina* (Jolly, 2001), *M. hecki* and *M. tonkeana* (Bynum et al., 1997; Bynum, 2002), *M. tonkeana* and *M. maura* (Supriatna, 1992; Evans et al., 2001; Schillaci and Froehlich, 2001), and *M. fuscata* and *M. cyclopis* (Kawamoto, 2005). There is also speculation that two recognized species in genus *Macaca*, *M. arctoides* and *M. munzala*, may be of hybrid origin resulting from previous hybridization events (Ackermann, 2010).

The inclusion of extant hybrid macaque skeletal elements would strengthen the comparative model for hominins given that there are known hybridization events within the

genus *Homo.* Current studies utilizing ancient DNA (aDNA) and fossil evidence have confirmed that anatomically modern *H. sapiens* possess small percentages of DNA that originated from other hominin species (Ackermann et al., 2019). Genome sequencing has confirmed that archaic populations of *H. sapiens* not only interacted with contemporary hominin species, but they were also able to produce fertile hybrid offspring. To date, this admixture has been documented between populations of *H. sapiens*, Neanderthals, and the Denisovans; however, there is speculation that other species will be added as additional specimens are recovered (Poinar, 1999; Jolly, 2001; Schillaci and Froehlich, 2001; Holliday, 2003; Ackermann, 2010; Green et al., 2010; Zinner et al., 2011; Ackermann et al., 2014; Smith et al., 2015; Ackermann et al., 2016; Kuhlwilm et al., 2016; Llorente et al., 2016; Sankararaman et al., 2016; Popadin et al., 2017; Prüfer et al., 2017; Slon et al., 2018). Given the previous results with identifying macaque morphological variation using GM techniques, it would be worthwhile to test whether hybrid species belonging to genus *Macaca* render similar results.

As stated previously, the main goals of paleoanthropology are to understand hominin evolutionary history and to elucidate the interactions between previous hominin species. Given the lack of available aDNA, the majority of hominin evolution must be determined through studying the morphology of newly discovered fossilized remains (von Cramon-Taubadel and Smith, 2012; Ackermann et al., 2019). Understanding how hybridization may impact the accurate identification of macaque skeletal elements could therefore improve our ability to recognize the morphological signatures of hybridity among hominin fossils (Ackermann, 2007; Ackermann and Bishop, 2009; Zinner et al., 2011; Ackermann et al., 2014; Ackermann et al., 2019). Ideally, implementing research using GM methods on hybrid macaque species would

provide insight into the taxonomic and phylogenetic classification of the genus, while also serving as a model for other variable primate species like hominins.

1.1 Hypotheses

This project is driven by the central question: can GM techniques be used to accurately identify and/or classify hybrid primate species based on variation in their skeletal morphology? To answer this question, two null hypotheses will be examined using a sample of *M. mulatta* that contains purebred and hybrid specimens. As stated previously, the goal of the current project is to create a more encompassing model using an analog nonhuman primate species by which hominin skeletal elements can be identified and classified regardless of whether their identity is known *a priori*. In addition to examining whether GM methods can render successful results while using a hybrid sample, I will also examine whether the skull or os coxa is more useful for inferring taxonomy. As a result, a third null hypothesis will be tested to help address previous arguments that state the skull is a better tool for making a successful identification in the field of paleoanthropology. After interpretation of the results, if the null hypotheses are rejected, then there is support for undertaking additional studies of this nature that examine hybridization as it relates to nonhuman primate and hominin skeletal morphology.

1.2 Null and Alternate Hypotheses

Ho1: Morphological variation among the hybrid specimens will not be distinct enough for GM methods to accurately allocate each specimen taxonomically.

Ha1: Morphological variation among the hybrid specimens will be distinct enough for GM methods to accurately allocate each specimen.

Ho2: The varying degrees of admixture represented in the sample will not display patterns in morphological variation when subjected to GM analyses.

Ha2: The varying degrees of admixture represented in the sample will display patterns of morphological variation when subjected to GM analyses.

Ho3: The skeletal morphology of the cranium will be able to allocate specimens more accurately in the sample than will the os coxa.

Ha3: The skeletal morphology of the ox coxa will be able to allocate specimens as well as or more accurately in the sample than does the cranium.

1.3 Expected Outcomes

Previous research has demonstrated the benefits of utilizing a quantitative method like GM on nonhuman primate species to examine variation in skeletal morphology. Given that past samples have consisted of purebred representative taxa, the results obtained from a hybrid sample have the potential to be different than those of their purebred counterparts. It is first necessary to understand the basics of hybridization as it pertains to a given species' evolution before one can try to determine how a hybrid sample would respond to GM analysis. A hybrid organism or species will occur when two distinct species are able to crossbreed and produce fertile offspring. The two parent species may belong to different genera or represent a subspecies within a larger species group. Both manmade and natural causes can result in the creation of a hybrid species, and once the reproductive barrier is removed, there are many

evolutionary consequences. These consequences can include a permanent merging of the two parent species, alteration of reproductive barriers between evolutionary distinct lineages, the creation of a novel phenotype unlike either parent species, formation of a hybrid zone, the extinction of one or both parent species, and the evolution of a new species (Anderson and Stebbins, 1954 Arnold, 1992; Holliday, 2003; Ackermann et al., 2006; Ackermann, 2010; Zinner et al., 2011; Ackermann et al., 2014; Ackermann et al., 2016).

The early stages of hybridization may produce offspring that appear to be similar to one or the other parent species, or they can possess a combination of traits from each species. At this point, a hybrid individual may be discerned from the parent species either visibly or genetically. After several generations, hybridization can also lead to introgression when the hybrid offspring interbreeds with members of the parent species. This process is known as backcrossing and will result in genes being reintroduced back into the parental lineages. As a result, the effects of hybridization may be harder to detect as members from each parent species and hybrid species can be physically indistinguishable from one another (Ackermann et al., 2010; Ackermann et al., 2016; Ackermann et al., 2019).

In the context of nonhuman primates, hybridization is not that uncommon and has been documented in roughly ten percent of known nonhuman primate species (Zinner et al., 2011; Ackermann et al., 2019). Researchers will commonly study hybridization in nonhuman primate species through examining their phenotypes and genotypes. The former will typically involve documenting traits that are visible to the naked eye, whereas the latter will involve studying the breakdown of mitochondrial DNA (mtDNA) and nuclear DNA of the specimen's genome (Ackermann 2010). Regardless of which method is used to examine a hybrid species,

studying the effects of hybridization in nonhuman primates can lead to important implications for the field of paleoanthropology. These include estimating when two parent species potentially experienced a hybridization event in the past that led to the creation of a hybrid species, studying how the effects of extant hybrid species may have existed in extinct ancestral species, and aiding in the identification or classification process of closely related nonhuman primate species (Arnold, 1992; Ackermann et al., 2014).

Rhesus macaques (*M. mulatta*) are known for their large geographic distribution that exceeds all other nonhuman primates. Populations of rhesus macaques are naturally found in Asia, ranging as far west as parts of Afghanistan, and extending to the eastern coast of China. Given the diversity of their habitats, a large amount of variation has been observed in rhesus macaque morphology that coincides with genetic differences that have been discovered (Fig. 2). In addition, given the popularity in using rhesus macaques as subjects for medical research, captive colonies of *M. mulatta* are commonly maintained that consist of individuals from different populations. Following a cease in exportation of Indian rhesus macaques, the most common population of *M. mulatta*, China became the main supplier of rhesus macaques to locations that housed colonies for research. As a result, captive colonies of rhesus macaques will typically have interbreeding recorded among individuals from different regional populations (Smith and McDonough, 2005).

Figure 2: Captive rhesus macaques to display phenotypic differences among living Chinese (left) and Indian (right) populations and among CT scans from the sample (left: Balouria, 2021; right: Sarangi, 2021).

Both captive and wild rhesus macaque populations have been observed to differ phenotypically, which includes the Chinese and Indian populations from the sample. Differences have been reported in their social behavior and organization, body size and length,

and physiology (Smith and McDonough, 2005). Given the difficulty in determining ancestry composition in the wild, a majority of phenotypic differences have been reported from captive rhesus macaque populations. That said, observational research in the wild indicates that on average, male Chinese rhesus macaques are larger than their Indian counterparts and tail-length is shorter in Indian rhesus macaque populations (Buck et al., 2021). In captivity, male Chinese rhesus macaques are on average heavier, longer, and taller than male Indian rhesus macaques. Conversely, female Indian rhesus macaques are significantly larger and heavier than Chinese rhesus macaques (Clarke and O'Neil, 1999). This pattern among captive and wild populations of males and females demonstrates the increased sexual dimorphism that is observed in Chinese rhesus macaque populations (Clarke and O'Neil, 1999; Buck et al., 2021).

Genetically, the mtDNA of Chinese rhesus macaques has four times the diversity of that seen in Indian rhesus macaques. In addition, there is higher genetic heterogeneity among Chinese rhesus macaques than their Indian counterparts (Smith et al., 2006). Additional research has shown that the mtDNA of Chinese and Indian rhesus macaques has as much genetic differences as that seen between separate purebred species of macaques. The mtDNA of Chinese rhesus macaques has been shown to be more similar to populations of other macaque species than to that of Indian rhesus populations. The nuclear genomes of Chinese and Indian rhesus macaques have also been observed to be divergent as seen in their allozymes, microsatellites, major histocompatibility complex loci, and SNPs (Kanthaswamy et al., 2009).

That said, the first and second null hypotheses take into consideration the potential effect of hybridization on the sample of *M. mulatta*. **Ho1** tests the notion that any potential

skeletal signatures of hybridization will not be distinct enough to discern between populations within the sample. Given that the sample contains both purebred parent populations and their hybrid offspring, it can be assumed that the *M. mulatta* specimens may be too morphologically similar to be differentiated. On the other hand, if the GM analyses can distinguish patterns among the parent and hybrid individuals in the sample, then there would be evidence to support **Ha1**. Similarly, **Ho2** will test whether there are any discernable patterns in the morphological variation that are sufficient enough to distinguish between the ancestry compositions in the hybrid sample. Following the rationale for **Ho1**, **Ho2** states that it should also be expected that the morphological variation of the different ancestry compositions would be too similar for GM methods to identify. Again, in the event that the GM analyses successfully groups the specimens based on the breakdown in their ancestry composition, there would be support for **Ha2**. Taken together, it would be expected that regardless of the results, the first and second hypotheses will either both be rejected, or there would be evidence to support both.

The third hypothesis is set apart from the other two in that instead of examining the potential taxonomic identification of the specimens, it will be focusing on the two skeletal elements being subjected to GM analysis. Sufficient research has shown the implications of equally utilizing skeletal elements from the crania and postcrania in paleoanthropology when available. The purpose of **Ho3** is to state that in the case of *M. mulatta*, purebred or hybrid, there will be more instances of the GM technique correctly allocating the specimen when examining the skull. Alternatively, **Ha3** states the opposite is true, where the os coxa will be as good as or more accurate than the skull is in allocating the macaque specimens in the sample.

Regardless of which hypothesis is supported by the results, the main goal of both **Ho3** and **Ha3** is to try and highlight any potential skeletal landmarks on the skull or os coxa that aided correct taxonomic identification of the specimens. This would further emphasize areas of either skeletal element that may possess visible signatures of hybridization that can be used to classify primate species more accurately.

Chapter 2: Materials and Methods

2.1 Sample

To address my research questions and hypotheses, I examined a sample consisting of two subspecies of *Macaca mulatta*, Indian and Chinese rhesus macaques, housed at the California National Primate Research Center (CNPRC) at the University of California, Davis. After four to five generations of interbreeding, the colony consists of a variety of macaques with differing levels of hybridization. There are still some purebred Indian *M. mulatta* and Chinese *M. mulatta* in the colony, and the admixture percentages ranged from 0.125-0.875% among the hybrid offspring. Individuals labeled with a CNPRC% of 0 represent purebred Indian *M. mulatta* and individuals with a CNPRC% of 1 represent the Chinese *M. mulatta* in the colony. That said, a specimen with a CNPRC% of 0.125 would represent a lineage breakdown of 0.125 from Indian *M. mulatta* and 0.875 from Chinese *M. mulatta,* whereas a specimen with a CNPRC% of 0.875 would indicate the opposite, and so on (Buck et al., 2021).

The sample consisted of 118 full-body medical computed tomography (CT) scans from adult *M*. *mulatta* housed at the CNPRC: twenty-one males and ninety-seven females. The admixture proportions in the dataset included six purebred Indian *M*. *mulatta*, three purebred Chinese *M. mulatta*, and 109 hybrid specimens with admixture percentages of 0.125, 0.25, 0.375, 0.5, 0.625, 0.75, and 0.875 (Table 1).

Table 1: Breakdown of sample (*n* = 118) by ancestry composition.

CNPRC%	0	0.125	0.25	0.375	0.5	0.625	0.75	0.875	1
Males	2	9	6	1	2	1	0	0	0
Females	4	46	17	11	4	3	8	1	3
Total	6	55	23	12	6	4	8	1	3

CNPRC% reflects the percentage of Chinese admixture: 0% represents a purebred Indian *M. mulatta* with no Chinese ancestry, 100% represents a purebred Chinese *M. Mulatta*.

Individuals were not chosen for inclusion in the sample if there was evidence of pathology or taphonomy in the CT scans that would interfere with data collection. All specimens were previously CT scanned at the CNPRC by appropriately trained staff. Many of the CT scans used in this thesis are freely available on request from the MorphoSource digital archive under the project name: The rhesus macaque admixture project (Project ID: 00000C291). Given the nature of the project, pedigree data have been recorded to indicate the ancestry compositions among individuals in the colony (Buck et al., 2021).

2.2 Data Collection

The CT scans were uploaded into the software program Avizo (Thermo Fischer Scientific, Waltham) and were virtually segmented to display only the skeletons of each individual using the available thresholding tools. After removing the other tissue types, the skull and pelvis were cropped from the skeleton of each specimen using segmentation tools. A bounding box was set around each scan, and the box was adjusted from multiple viewpoints to crop the specimen in all dimensions. Each scan was zoomed in far enough to only display the skull and os coxa per

individual, while ensuring that the entire bone was clearly visible. All files were saved in the .obj format as surface models to use with the landmarking process. Next, I used the Stratovan Checkpoint software program to assign landmark coordinates to capture the overall variation in shape. After uploading the cropped files of the skulls and pelves, I placed landmarks along the left side of each bone in the same order for each specimen in the sample. All landmarking was completed one bone at a time and for one individual at a time before proceeding on to the next specimen. That is, one specimen's skull was fully labeled with landmark coordinates, then the respective pelvis, and so on. A total of forty-nine landmarks were placed on the cropped CT scans per individual, twenty-nine for the skull and twenty for the pelvis (See Tables 2-3 and Figs. 3-4 for details). Landmark data were exported as .csv files for further analyses.

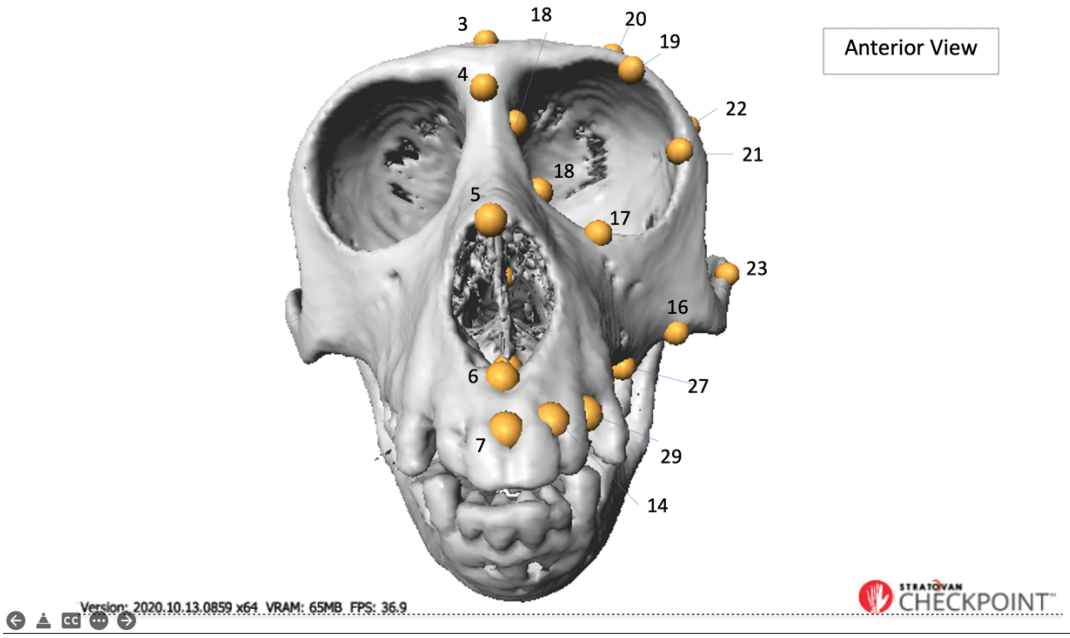
All landmarking coordinates were placed by the author, and therefore interobserver error was not assessed. Intraobserver error was addressed by undertaking a trial run in the landmarking process. Five specimens were selected, for a total of five skulls and five pelves, and the landmark coordinate sets were labeled three times each for each individual. A total of fifteen skulls and fifteen pelves were labeled and then uploaded into the software program MorphoJ (Klingenberg, 2011) to examine whether the landmark coordinates clustered after a Principal Components Analysis (PCA). Low intraobserver error was confirmed via the fact that each set of landmark trials clustered tightly per individual on the PC plot.

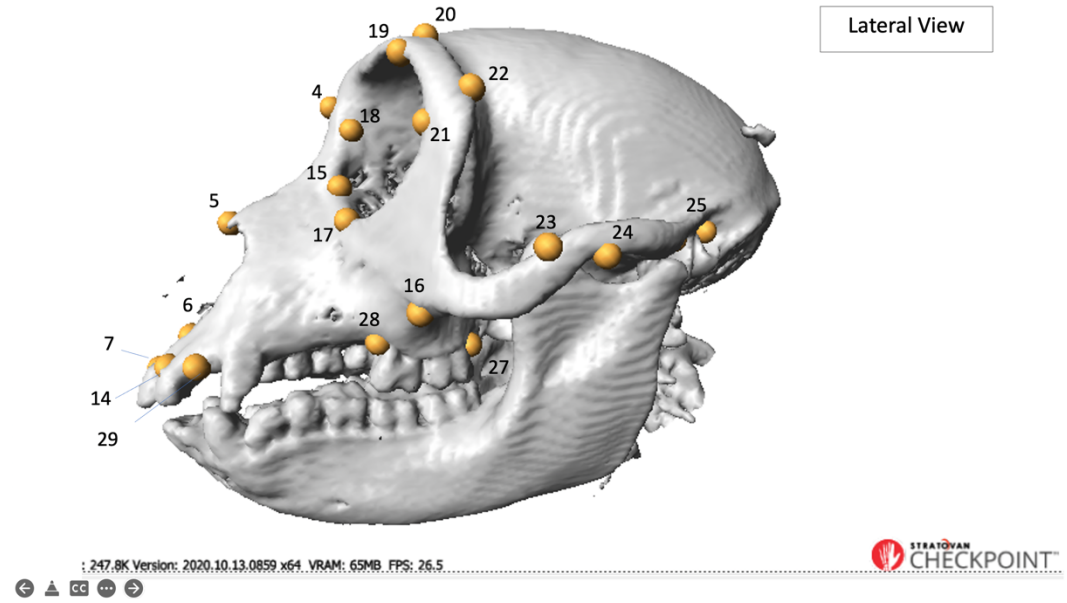
With no visible discrepancies in the trial run, the landmarking process for every specimen was completed. Once all landmarks were placed, the data were exported as .csv files and then transferred into .txt files to carry out further analyses in Morphologika2 and MorphoJ.

The landmark data for each bone were separated into two .txt files, one for the skull and another for the pelvis.

Table 2: Anatomical locations for cranial landmarks (adapted from Kenyon-Flatt, 2020). See Figure 1 for landmark positions.

#	Landmark	#	Landmark
1	Inion	16	Inferior Zygomaxillary
2	Bregma	17	Superior Zygomaxillary
3	Supra-glabella	18	Dacryon
4	Nasion	19	Mid-torus Inferior
5	Rhinion	20	Mid-torus Superior
6	Subspinale	21	Medial Frontozygomatic Suture
7	Prosthion	22	Lateral Frontozygomatic Suture
8	Incisivion	23	Zygo-temporal Superior
9	Palatal Suture	24	Zygo-temporal Inferior
10	Vomer	25	Porion
11	Spheno-basion	26	Glenoid
12	Basion	27	Distal M3
13	Opisthion	28	M1-M2
14	Prosthion 2	29	Premax-Maxillary Inferior
15	Premax-maxillarysupperior		


Anterior View
3
18
20
19
4
22
21
18
5
17
23
16
6
27
7
29
14
Version: 2020.10.13.0859 x64 VRAM: 65MB FPS: 36.9
STRATOVAN CHECKPOINT


Lateral View
20
19
22
4
18
21
15
5
25
17
23
24
16
6
28
7
27
14
29
: 247.8K Version: 2020.10.13.0859 x64 VRAM: 65MB FPS: 26.5
STRATOVAN CHECKPOINT

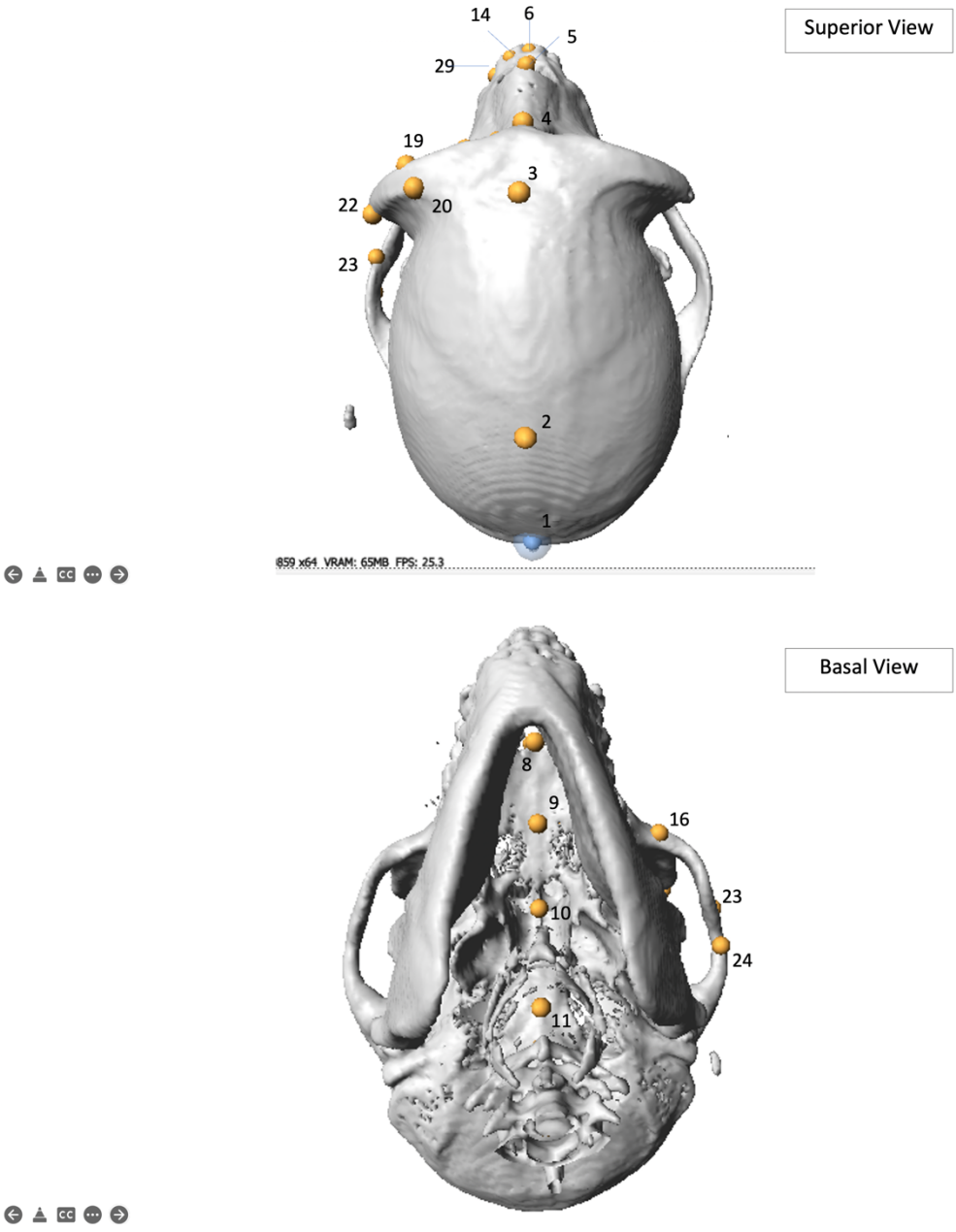
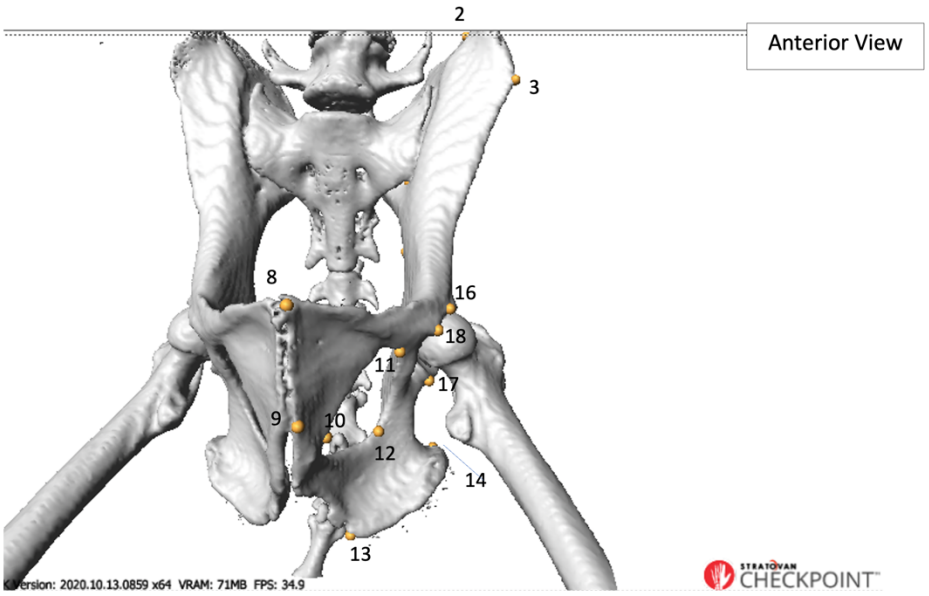
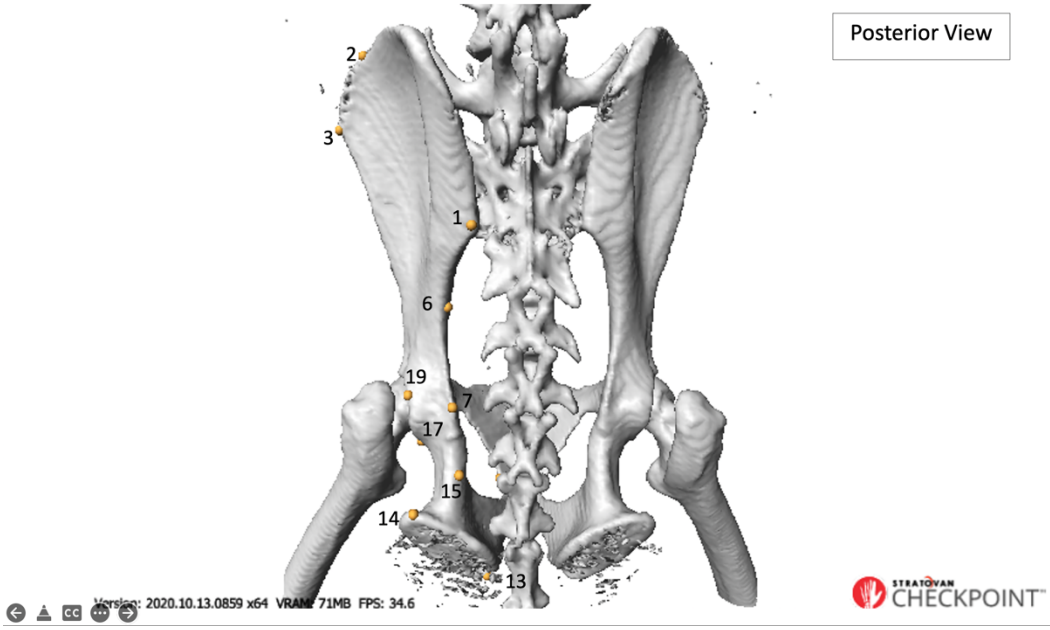


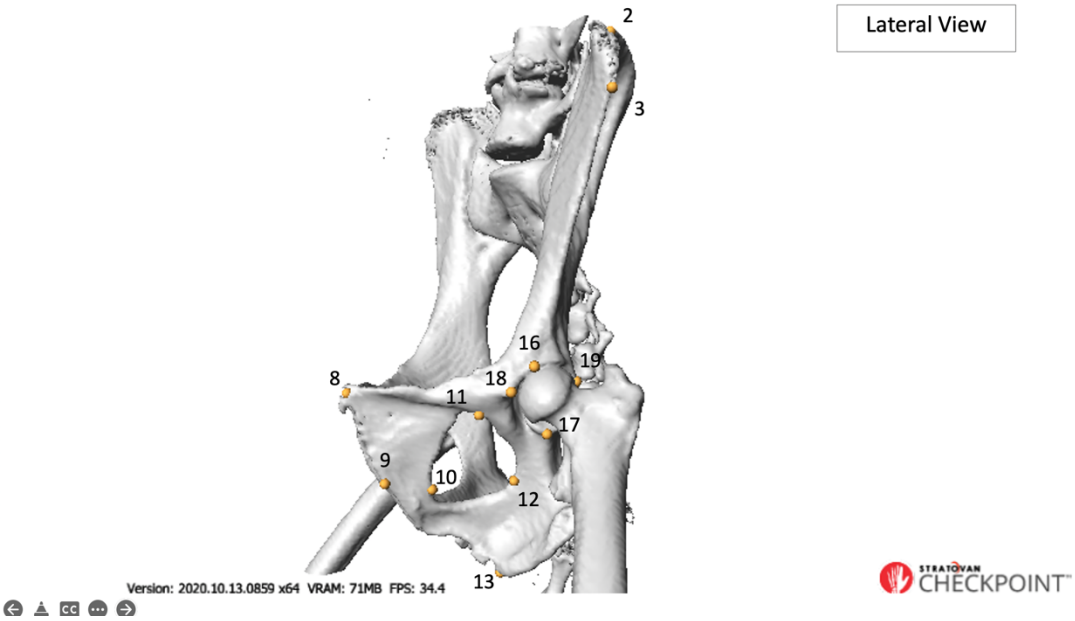
Figure 3: Cranial landmarks. Four views (A: Anterior, B: Lateral, C: Basal, D: Superior) of a macaque skull showing anatomical positions of 29 landmarks.

Table 3: Anatomical locations for os coxa landmarks (adapted from Kenyon-Flatt, 2020). See Figure 2 for landmark positions.

#	Landmark	#	Landmark
1	Iliac Crest Medial	11	Obturator Superior
2	Iliac Crest Superior	12	Obturator Posterior
3	Iliac Crest Lateral	13	Ischial Tuberosity Anterior
4	Auricular Superior	14	Ischial Tuberosity Posterior
5	Auricular Inferior	15	Ischium Posterior
6	Sciatic Notch	16	Acetabulum Superior
7	Ischial Spine	17	Acetabulum Inferior
8	Pubic Symphysis Superior	18	Acetabulum Anterior
9	Pubic Symphysis Inferior	19	Acetabulum Posterior
10	Obturator Anterior	20	Acetabulum Center


Anterior View
2
3
8
16
18
11
17
9
10
12
14
13
Version: 2020.10.13.0859 x64 VRAM: 71MB FPS: 34.9
STRATOVAN CHECKPOINT


Posterior View
2
3
1
6
19
7
17
15
14
13
Version: 2020.10.13.0859 x64 VRAM: 71MB FPS: 34.6
STRATOVAN CHECKPOINT



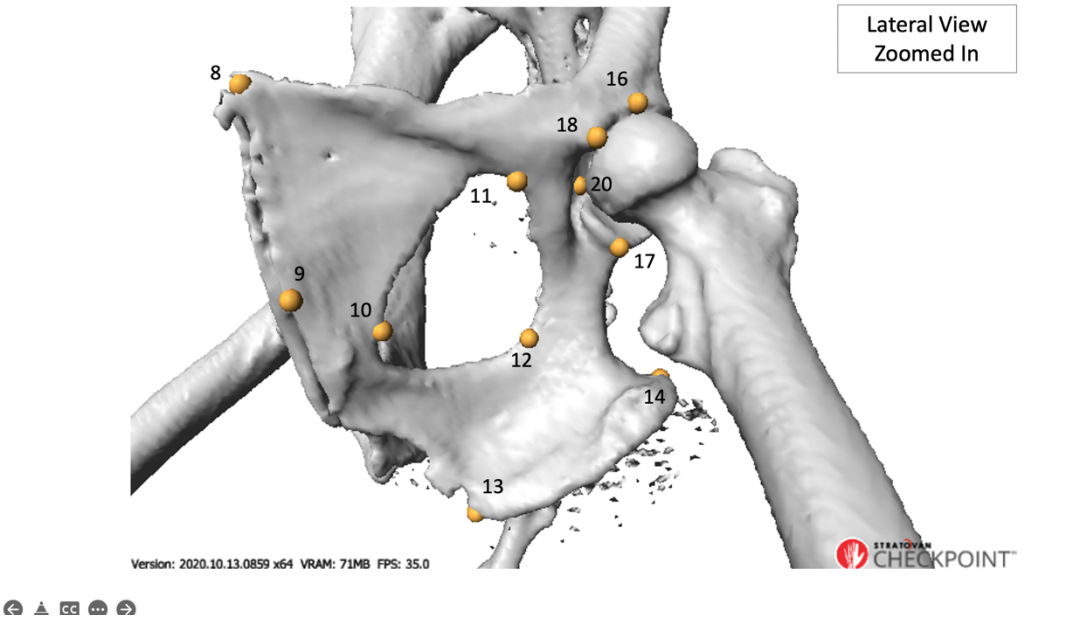


Figure 4: Os coxa landmarks. Four views (A: Anterior, B: Posterior, C: Lateral, D: Lateral Zoomed In) of a macaque pelvis showing anatomical positions of 20 landmarks.

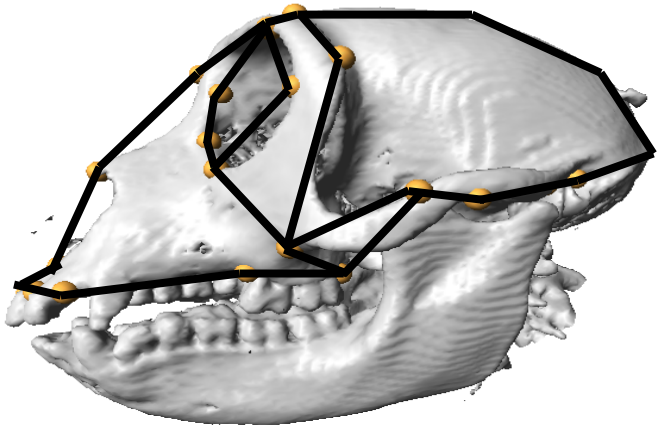


Figure 5: Wireframe of landmarks on the skull in lateral view.

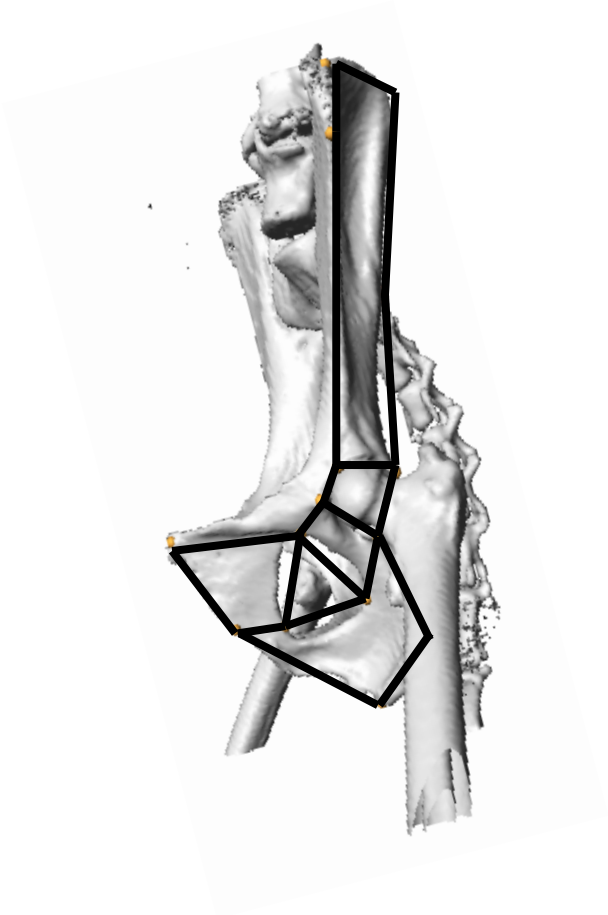


Figure 6: Wireframe landmarks on the os coxa in lateral view.

2.3 Data Analyses

Following the landmarking process, the two .txt files containing the landmark data from the skulls and pelves were imported into the software programs Morphologika2 and MorphoJ. Statistical analyses were carried out separately for the skull and pelvis. Morphologika2 was used during the preliminary assessment of the datasets, where a Procrustes analysis and principal component analysis (PCA) were conducted for each dataset. Here, visualization of the wireframes allowed for examination of variation in the datasets and shape changes along the PC axes. Next, the datasets were imported into MorphoJ, where statistical tests performed included Procrustes ANOVA, Regression, PCA, Canonical Variates Analysis (CVA), and Discriminant Function Analysis (DFA).

2.3.1 Procrustes ANOVA

Previous research that used GM on purebred macaque species hypothesized that sexual dimorphism may have a confounding effect when investigating each species' skeletal morphology. Results indicated that sexual dimorphism did influence the degree of variation among the skeletal elements, which indicated that it would be necessary to control for sex when using additional analyses to discriminate between different populations belonging to the same genus (Kenyon-Flatt et al., 2020; Kenyon-Flatt, 2020). A Procrustes ANOVA was conducted on each dataset to determine whether sexual dimorphism influenced the variation in skeletal morphology of the skull and os coxa within the hybrid sample. Results from a Procrustes ANOVA would help determine whether there was an interaction between two categorical variables, sex and species, which would indicate if either bone exhibited strong effects caused

by sexual dimorphism. A significant value (p-value ≤ 0.05) suggests that there is an interaction between the categorical variables and a p-value ≥ 0.05 suggests that there is not an interaction (Zelditch et al., 2004).

2.3.2 Regression

Knowing that sex was a confounding variable, it was necessary to control for its effects so that a categorical variable could be distinguished in the sample. A multivariate regression was conducted to help explain how several variables, in this case shape, are linearly related to each other (Kenyon-Flatt, 2020). In MorphoJ, the regression was performed by assigning independent and dependent variables to the data. The dependent variable was assigned to the Procrustes coordinates and the independent variable was assigned as the centroid size. The regression measures the degree to which independent variables and dependent variables are linearly related, which helps indicate the vertical distance between specimens and the regression line. This data is displayed as regression residuals, which are later imported into a PCA and CVA for additional analyses.

2.3.3 Principal Component Analysis (PCA)

A principal component analysis (PCA) was the first method used to observe the shape diversity in the dataset. The PCA simplifies differences among individuals, which aids in the visualization of variation among individuals in the sample. Given the complexity of the raw geometric shape variables, a PCA can simplify any existing patterns of shape variation and make them easier to interpret. Procrustes shape variables are replaced with Principal Components

(PCs), which represent linear combinations of the variables that are independent from each other. A PCA plot and statistical output table are generated and can show whether there are systematic patterns of variation from the sample. PCA results are displayed as eigenvalues with respective percentages of variance, and each eigenvalue will be explained by an eigenvector. An eigenvalue will explain the amount of variation by indicating how much variance in the data exists in a single direction (Zelditch et al., 2004). The highest eigenvector represents PC1, with the next highest representing PC2, and so on. Each eigenvector corresponds to the principal components and the given eigenvalues will correspond to the percent variance that is explained by the principal components (Kenyon-Flatt, 2020).

After the PCA was conducted for each dataset in MorphoJ, the patterns represented by each ancestry composition were difficult to visualize because of the quantity of categorical groups. A second set of PCAs were conducted that simplified the ancestry compositions represented in the sample. More specifically, purebred specimens were relabeled as “FullI” and “FullC”, representing purebred Indian rhesus macaques and Chinese rhesus macaques respectively. Specimens with a 0.5 CNPRC% were labeled as “Half”. Specimens with the CNPRC% of 0.125, 0.25, and 0.375 were grouped together and labeled as “MostlyI” to indicate an ancestry composition mainly consisting of an Indian rhesus macaque. Specimens with the CNPRC% of 0.625, 0.75, and 0.875 were grouped together and labeled as “MostlyC” to indicate an ancestry composition mainly consisting of a Chinese rhesus macaque. The newly created group designations permitted a PCA to display patterns among five broader groups to aid in visualization, instead of the former group designations. After the updated PCAs were

performed, PC1 scores were regressed against centroid size for each dataset to determine what percentage of the variation along PC1 is accounted for by size.

2.3.4 Canonical Variates Analysis (CVA)

The second method used to observe morphological variation was a canonical variates analysis (CVA), which can describe patterns of variation among groups in a sample. The CVA is different from a PCA because the CVA interprets variation among groups, whereas the PCA interprets variation among individuals in a given sample. A CVA will create a new coordinate system consisting of canonical variates (CVs), which determines the scores on those axes for each specimen in a sample. CVs are linear combinations of the existing variables that are mutually orthogonal. A CVA can describe differences in group means and utilizes patterns of within-group variation to scale the axes of the newly created coordinate system. A CVA plot will be generated that displays eigenvalues like a PCA, although CV1 would indicate which direction the group is most effectively discriminated. In the case of a CVA, the number of groups (*n*) will be generated as *n*-1 CV scores (Zelditch et al., 2004).

Another way the CVA differs from a PCA is that the former statistical test will describe any group differences based on previously known group assignments. In the case of the current sample, the group assignments represented the specimen's admixture identifications. The results obtained from a CVA will help examine the first and second hypotheses by determining whether groups can be identified based on patterns in morphological variation. The CVA plots assess whether there are morphological signatures in the skull and os coxa that created patterns by which the CVA could assess group variation.

The CVA was conducted in MorphoJ for each dataset and the regression residuals were selected as the raw data so that the effect of sex was removed from the data. The ancestry compositions were selected as the classifier so that the CV scores represented the groups utilized in the sample.

2.3.5 Discriminant Function Analysis (DFA)

A discriminant function analysis (DFA) was conducted in MorphoJ for each dataset to first classify specimens from the sample according to sex to further investigate whether sexual dimorphism was a confounding variable. The DFA was used as a statistical procedure to classify unknown specimens and return the probability of their classification into a specific group (in this case, sex). The DFA was used in this context because the sex of each specimen from the sample was known *a priori*, and it was determining the probability of a correct/incorrect classification for each individual based on sex. The DFA was performed using cross-validation and assessed how the results would compare to an independent dataset. This is a predictive model that can estimate the accuracy of each bone in labeling the specimen to its correct sex. After conducting a DFA on sex in the sample, a secondary DFA was performed that specifically examined the ancestry compositions of the sample that were also known *a priori*. This helped address the first two hypotheses that investigated whether the variation in skeletal morphology of the skull and os coxa were distinct enough for a DFA to accurately predict the species of each specimen. If the accuracy of each prediction from the DFA are high, then there is an adequate signal in each bone that could be used to help identify the given species. If the accuracy is low, then the variation was so small that the DFA will incorrectly classify the specimens in each

group. After comparing the accuracy of the skull and os coxa among groups, the third hypothesis can be addressed to determine which bone is able to more accurately allocate the specimens.

Chapter 3: Results

3.1 Procrustes ANOVA Results

The skull and os coxa reported significant *p*-values, which stated differences existed in the bones caused by sex in the sample (Table 4). Similar to the research on purebred macaque species, the hybrid sample also suggested it was necessary to control for sex as a confounding variable, and thus subsequent analyses took this into account. Conversely, the skull and os coxa reported non-significant *p*-values when determining whether there were differences caused by the hybridization composition (Table 5).

Table 4: *p*-values reported from the Procrustes ANOVA investigating the interaction between sex and species.

Bone	*p*-value
Skull	<.0001
Os Coxa	<.0001

Table 5: *p*-values reported from the Procrustes ANOVA investigating the interaction between ancestry composition and species.

Bone	*p*-value
Skull	0.9550
Os Coxa	0.0680

3.2 PCA Results

Two sets of principal components analyses (PCA) were performed for each dataset. The first set of PCAs were performed in Morphologika2 and the second were performed in MorphoJ. In Morphologika2, a PCA was the first tool utilized to visualize patterns in the data and to determine if there were any areas on the skull or os coxa where morphological variation was meaningful. In Morphologika2, you can use the PCA plot to track changes in variation via the wireframe model along the axes. The principal component (PC) 1, which accounts for 15.5% of the variance, tracks changes in size of the skull and the length of the face (see Table 6). PC2, which accounts for 11.2% of the variance, tracks changes in the upper midline of the skull and the zygo-temporal area (see Table 6). Along PC1, there was an increase in the overall cranium height and a decrease in the overall cranium length as you moved from negative to positive. In addition, along PC1, the facial shape was longer on the negative axes and flatted as you move towards the positive axis. Regressing PC1 against centroid size for the cranial dataset revealed that 10.7% of the variation along this axis is accounted for by size. Along PC2, shape differences were captured in the area of landmark two and twenty-three as you move from the negative axis towards the positive axis. More specifically, this translates to variation in the position of bregma along the superior midline of the skull and the superior portion of the zygo-temporal area. Moving towards the positive axis, bregma shifts forward on the skull and zygo-temporal superior shifts upwards on the skull.

In MorphoJ, the PCA was used to visualize two different divisions of the skull dataset, first considering differences by sex separately (see Fig. 7) and then considering differences by hybridization composition with sex combined per group (see Fig. 8). Males and females were

differentiated by color along PC1 and PC2 and were observed closely clustered together. PC1 roughly separates females and males, with males clustering more towards the negative end of the axis and females more towards the positive, although there is a fair degree of overlap (Fig. 7). Different color coding of the same PC1 and PC2 plot by ancestry compositions revealed no distinct cluster patter, although the hybrid specimens showed much larger ranges of variation than did the purebred specimens (Fig. 8). Overall, the dataset consisting of the skulls displayed no distinct separation by either sex or ancestry composition as seen in Figures 7 and 8. This indicates that the skulls of the hybrid specimens are slightly more variable than those of the purebred specimens, but that the range of variation of the purebreds is within that of the hybrids.

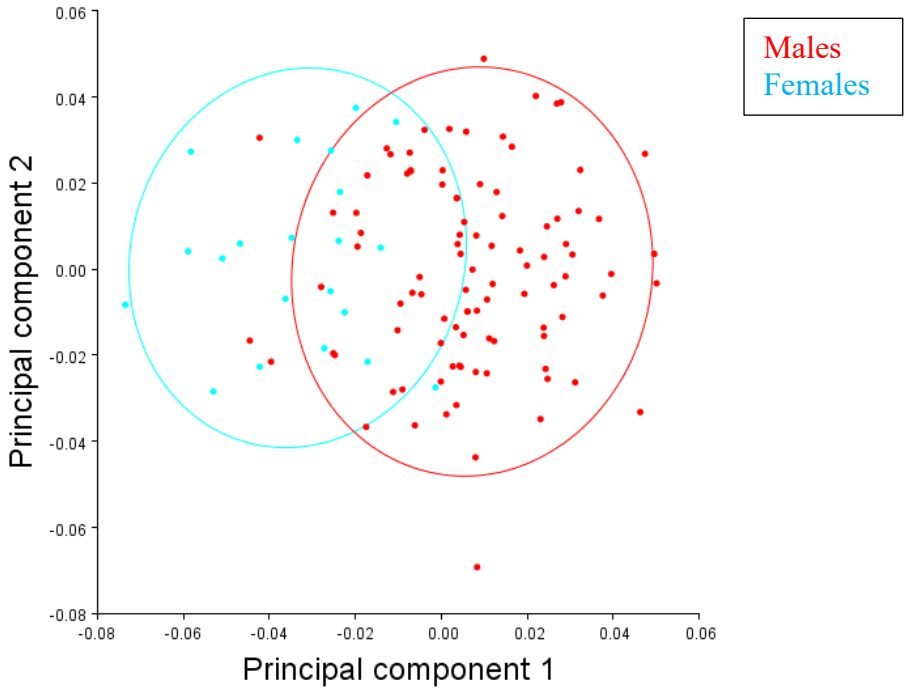


Figure 7: Principal component analysis results by sex for the skull where males are represented by blue and females are represented by red.

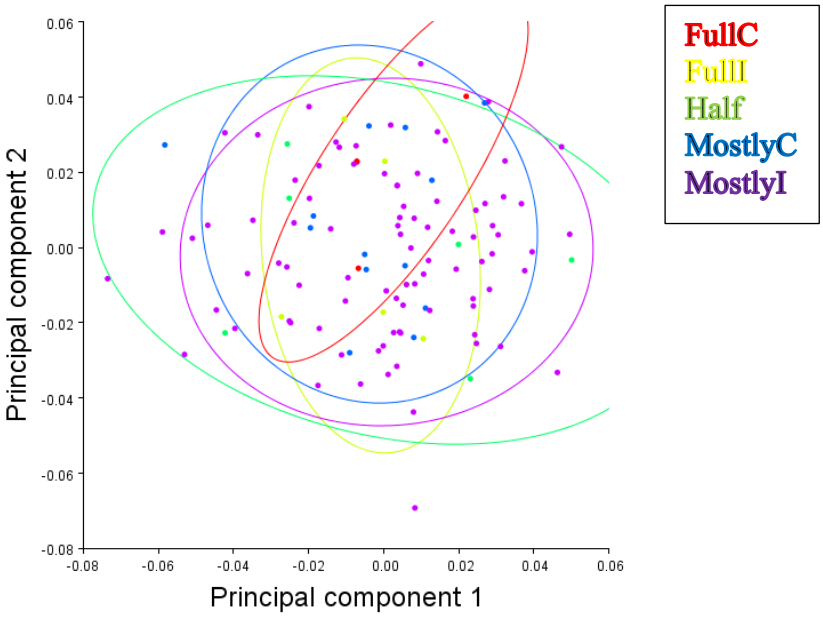


Figure 8: Principal component analysis results by ancestry composition with sex combined per group for the skull. Hybrid compositions are separated by color.

Table 6: Principal component analysis results provided as significant eigenvalues, percent variance, and cumulative percentage of variance for the skull.

	Eigenvalue	% Variance	Cumulative %
1	0.00061474	15.439	15.439
2	0.00047494	11.180	27.367
3	0.00040906	10.273	37.640
4	0.00022741	5.711	43.352

For the pelvis dataset, the Morphologika2 PCA showed that PC1, which accounts for 14.4% of the variance, tracks changes in the pubic symphysis and iliac crest of the os coxa (Table

7). PC2, which accounts for 11.2% of the variance, also tracks changes in the pubic symphysis and iliac crest of the os coxa (Table 7). Along PC1, there was a shape difference along the pubic symphysis as you move from the negative axis to the positive axis. The superior and inferior pubic symphysis landmarks transition outward and downward away from the remainder of the os coxa. In addition, the iliac crest landmarks shift cranially as you move from the negative axis to the positive axis. Conversely, along PC2, the variation of the pubic symphysis tracks in the opposite direction as you move towards the positive axis. The pubic symphysis landmarks transition cranially and medially as you move from the negative axis to the positive axis. In addition, along PC2, the landmarks of the iliac crest transition cranially as you move from the negative axis to the positive axis as seen in PC1. Regressing PC1 against centroid size for the pelvis dataset reveals that size accounts for only 3.5% of the variation along the axis.

In MorphoJ, as seen with the skull dataset, the PCA plot for the os coxa dataset was visualized in two different ways. The first consideration was differences by sex separately (see Fig. 9) and then differences by hybridization composition with sex combined per group (see Fig. 10). Males and females were somewhat separated along PC1, with females towards the negative end of the axis and males towards the positive end, but the male range almost entirely overlapped part of that of the females (Fig. 9). The hybrid compositions showed almost total overlap by groups along PC1 and PC2, with the purebred Indian and purebred Chinese macaques somewhat separated from each other and all the hybrids overlapping the bulk of their ranges of variation (Fig. 10). Overall, the dataset consisting of the pelves displayed minimal variation among the ancestry composition groups as seen in Figures 9 and 10. This

indicates that the pelves of purebred and hybrid specimens, regardless of sex, were overall morphologically fairly similar to one another.

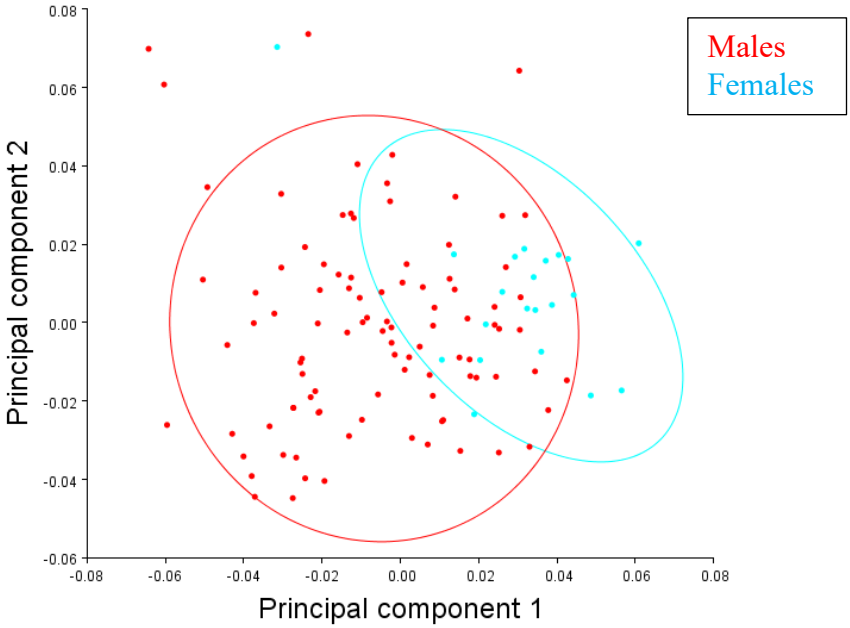


Figure 9: Principal component analysis results by sex for the os coxa where males are represented by blue and females are represented by red.

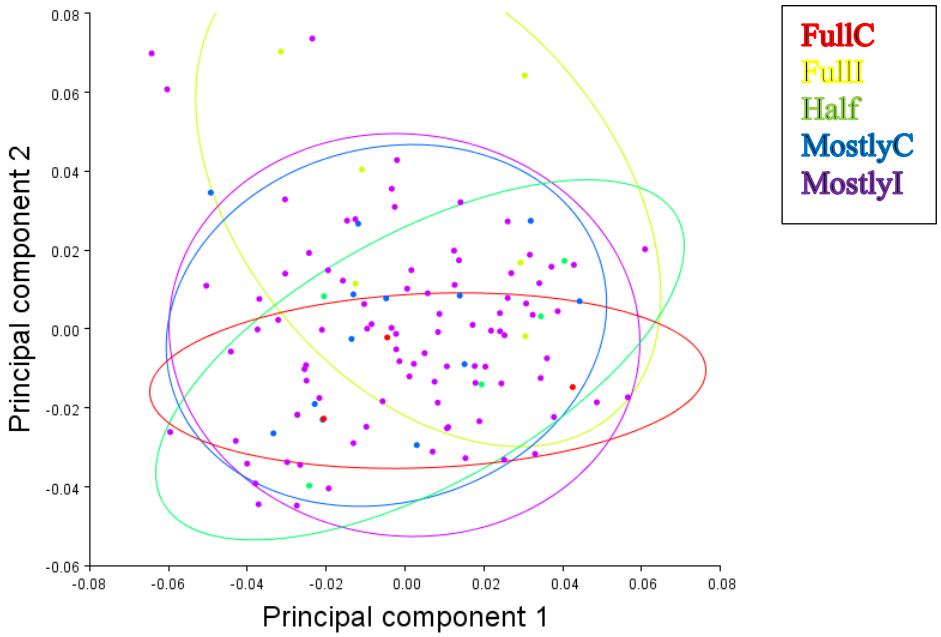


Figure 10: Principal component analysis results by ancestry composition with sex combined per group for the os coxa. Hybrid compositions are separated by color.

Table 7: Principal component analysis results provided as significant eigenvalues, percent variance, and cumulative percentage of variance for the os coxa.

	Eigenvalue	% Variance	Cumulative %
1	0.00075664	14.040	14.040
2	0.00060253	11.180	25.220
3	0.00043771	8.122	33.342
4	0.00037073	6.879	40.222
5	0.00034578	6.416	46.638
6	0.00030924	5.738	52.376

3.3 CVA Results

Two canonical variate analyses (CVA) were conducted in MorphoJ that grouped the specimens based on their ancestry composition while combining sexes. The CVA for the skull dataset revealed group differences among the purebred Chinese, mostly Chinese, and half hybrid macaques. The purebred Indian and mostly Indian macaques clustered together and were indistinguishable from each other (Fig. 11). Canonical variate (CV) 1, which accounts for 57% of the variance among the groups, tracked shape differences at the posterior end of the skull, maxilla, and the occipital orbit (Table 8). More specifically, landmarks six, seven, and fourteen at the anterior end of the maxilla displayed shape differences, as did the landmarks surrounding the occipital orbit (four, seventeen, nineteen, and twenty). CV2, which accounts for 22% of the variance among groups, tracked shape differences at bregma (landmark two), rhinion (landmark five), and the zygomatic area (landmarks sixteen and twenty-four) (Table 8).

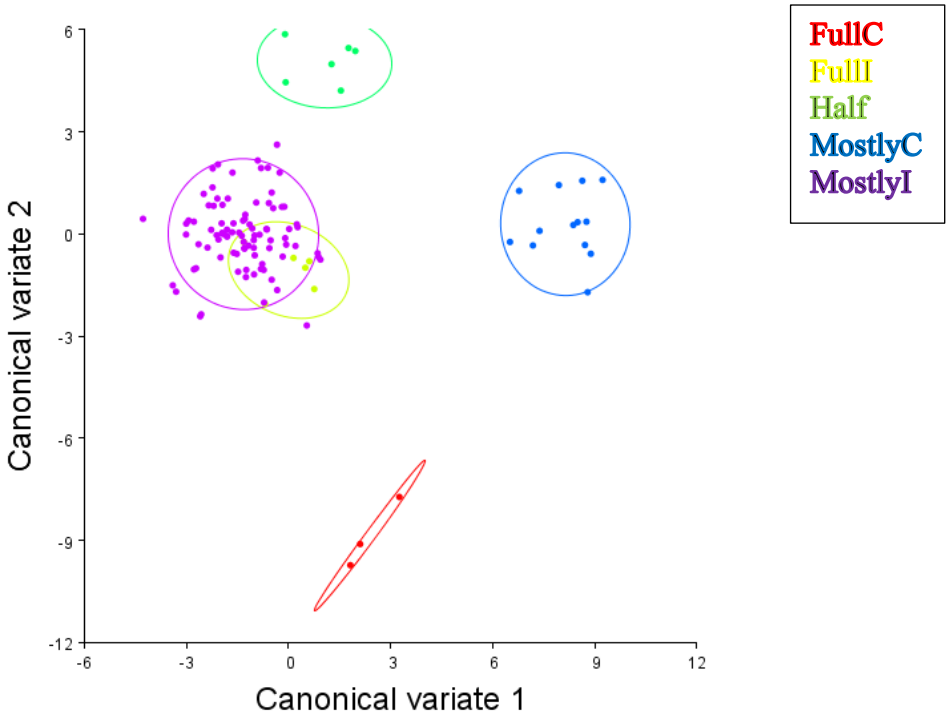


Figure 11: Canonical variate analysis results by ancestry composition for the skull.

Table 8: Canonical variate analysis results provided as significant eigenvalues, percent variance, and cumulative percentage of variance for the skull.

	Eigenvalue	% Variance	Cumulative %
1	9.21978017	56.668	56.668
2	3.51147122	21.583	78.250
3	2.12296742	13.048	91.299
4	1.41566367	8.701	100.000

The CVA for the pelvis dataset revealed group differences between the purebred Indian and purebred Chinese macaques, with the former closer to the negative axis and the latter distinguished as the group at the furthest point along the positive axis. All three hybrid

compositions, the half, mostly Indian, and mostly Chinese, were clustered together in between the two purebred groups. The CVA of the pelves revealed clearer separation between the purebred and hybrid groups than did that of the CVA of the skull (Fig. 12). Both CV1 and CV2 tracked shape differences in nearly all twenty landmarks to an extent, but only the most drastically different landmarks were reported. CV1, which accounts for 47% of the variance among the groups, tracked larger shape differences in the iliac crest, pubic symphysis, obturator, and ischial tuberosity (Table 9). More specifically, landmarks one, three, and four at the iliac crest, landmarks eight and nine of the pubic symphysis, landmarks ten and twelve surrounding the obturator foramen, and landmarks thirteen and fourteen of the ischial tuberosity displayed the most distinct variation. CV2, which accounts for 23% of the variance among the groups, tracked shape differences similarly to CV1 at the iliac crest and pubic symphysis (Table 9). In addition, CV2 tracked changes at landmarks fourteen and fifteen at the ischium, and at landmarks sixteen to nineteen surrounding the acetabulum.

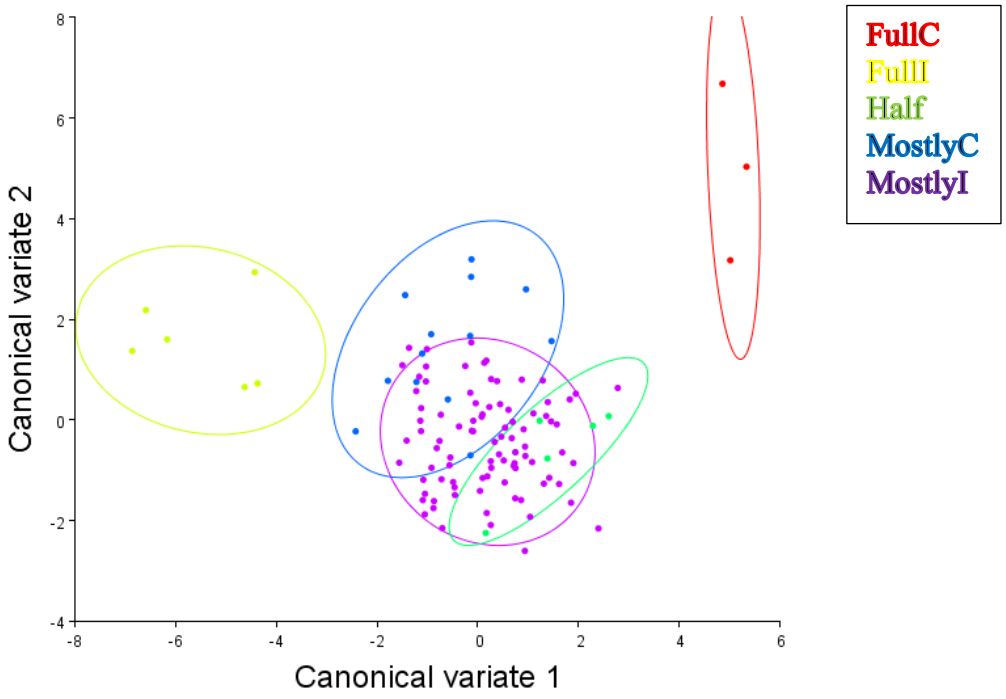


Figure 12: Canonical variate analysis results by ancestry composition for the os coxa

Table 9: Canonical variate analysis results provided as significant eigenvalues, percent variance, and cumulative percentage of variance for the os coxa.

	Eigenvalue	% Variance	Cumulative %
1	2.47297681	47.103	47.103
2	1.18503779	22.572	69.675
3	0.92856356	17.687	87.361
4	0.66354007	12.639	100.000

3.4 DFA Results

Discriminant function analysis (DFA) was performed based on two classifiers for each bone. The first classifier separated the groups by sex, whereas the second classifier separated groups by their ancestry compositions. The DFA of the dataset consisting of the skulls using the

classifier of sex had a rate of correct classification ranging from 81% to 95%, for males and females respectively. The DFA of the pelvis dataset using the classifier of sex had a correct classification ranging from 90% to 97%, for males and females respectively. When using the classifier for ancestry composition, each group was subjected to a cross-validation test to determine what percentage of the individuals could be correctly classified back to their own group. In general, the mostly Indian and mostly Chinese groups had the highest accuracy being classified correctly among the skull and os coxa. All combinations of the cross-validation test that consisted of the mostly Indian or mostly Chinese as one group had a higher accuracy than those without one of the two groups. The purebred Indian and purebred Chinese groups had the lowest accuracies reported from the cross-validation tests. In addition, the overall rate of accurately classifying individual was higher when using the os coxa than when using the skull.

Table 10: Cross-validated DFA results for the skull with the ancestry composition classifier reported as percent correctly identified.

Groups Compared	% Correctly Classified
Purebred Chinese or Purebred Indian	22%
Purebred Chinese or Half	22%
Purebred Chinese or Mostly Chinese	81%
Purebred Chinese or Mostly Indian	77%
Purebred Indian or Half	25%
Purebred Indian or Mostly Chinese	79%
Purebred Indian or Mostly Indian	64%
Half or Mostly Chinese	47%
Half or Mostly Indian	63%
Mostly Chinese or Mostly Indian	80%

Table 11: Cross-validated DFA results for the pelvis with the ancestry composition classifier reported as percent correctly identified.

Groups Compared	% Correctly Classified
Purebred Chinese or Purebred Indian	55%
Purebred Chinese or Half	33%
Purebred Chinese or Mostly Chinese	81%
Purebred Chinese or Mostly Indian	97%
Purebred Indian or Half	83%
Purebred Indian or Mostly Chinese	58%
Purebred Indian or Mostly Indian	87%
Half or Mostly Chinese	84%
Half or Mostly Indian	71%
Mostly Chinese or Mostly Indian	72%

Chapter 4: Discussion and Conclusions

The goals of this thesis were 1) to determine whether GM methods could accurately identify a sample of closely related hybrid nonhuman primate populations based on the variation in their skeletal morphology, 2) to create a better analogous model using a nonhuman primate species that could be used with hominin species, and 3) examine and/or compare the usefulness of the skull and os coxa in inferring taxonomy. Results from the statistical analyses provided a consensus that the morphological variation within the sample was not distinct enough to be able to reliably distinguish between or among purebred or hybrid specimens. The first and second null hypotheses were generally supported, whereas the third null hypothesis was neither supported nor refuted based on the interpretation of the results.

Going into the project, the expected outcomes were based on previous research, and as a result, it was assumed that the null hypotheses would be supported after interpreting the results. As stated previously, the null hypotheses were as follows:

Ho1: Morphological variation among the hybrid specimens will not be distinct enough for GM methods to accurately identify each specimen taxonomically.

Ho2: The varying degrees of admixture represented in the sample will not display patterns in morphological variation when subjected to GM analyses.

Ho3: The skeletal morphology of the cranium will be able to identify specimens more accurately in the sample than will the os coxa.

Given that the sample consisted of purebred and hybrid individuals from two populations of the same species, it was entirely possible that the results could be similar to, or differ from, previous research that has examined macaque morphological variation via GM

methods. In this case, both purebred populations in the sample were *M. mulatta*, and therefore, it was assumed that any variation among the skeletal elements would be minimal. Conversely, since a large portion of the sample consisted of *M. mulatta* with varying degrees of ancestry compositions, it was possible that their morphology would possess traits from each purebred population. Generally, hybrid organisms will display random combinations of traits from each parent species. However, since these two populations were so closely related, the potential for any drastic changes in morphology over subsequent generations was thought to be unlikely. After taking that into consideration, the first and second null hypotheses stated that the morphological variation in the sample would not be distinct enough for GM methods to register at the individual- or group-level. Results indicated that this was true, which refuted the first and second alternative hypotheses. **Ha1** stated that the specimens, hybrid or purebred, would be correctly identified based on pronounced morphological variation. Similarly, **Ha2** stated that patterns of morphological variation among groups would be prominent enough to discern between ancestry compositions in the sample. As stated in the introduction, the expectation was that the evidence would neither support both **Ho1** and **Ho2**, or vice versa, both would be refuted. This ended up being true, in that both null hypotheses were supported by the results.

The third hypothesis was also created based on previous research, although the results could have supported the null (**Ho3**) or alternate (**Ha3**) hypotheses. There is a longstanding debate in the field of paleoanthropology that argues which skeletal elements are most effective at making an accurate identification and classification. There are those who suggest that the cranium and its associated skeletal elements are more reliable (e.g., Asfaw et al., 2002;

Roseman, 2004; Ackermann, 2007; Fleagle et al., 2010; Smith and von Cramon-Taubadel, 2015), and those who favor the skeletal elements of the postcrania (e.g., Young, 2008; Lycett and von Cramon-Taubadel, 2013; von Cramon-Taubadel and Lycett, 2014; Kenyon-Flatt, 2020; Kenyon-Flatt et al., 2020). There has been considerable progress made that shows the importance of utilizing any available skeletal elements when identifying and classifying known or unknown specimens. That said, the third hypothesis sought to examine whether the skull or os coxa would display more pronounced morphological variation in the sample when analyzed via GM methods. If the results determined that the skull was a more accurate indicator of a specimen's taxonomic identification, then **Ho3** would be supported. Conversely, if the os coxa accomplished this task and refuted the null hypothesis, then there was support for **Ha3**. Either version of the third hypothesis would have been notable, as the main purpose was simply to examine if there were any significant landmarks that may have indicated higher amounts of morphological variation. This would have indicated potential anatomical locations of the skull or os coxa that may have had any morphological signatures of hybridization that could have been useful in distinguishing individuals from the sample. Interpretation of the results stated that GM methods were unable to recognize anatomical locations of either bone that were able to reliably distinguish individuals in the sample of *M. mulatta* based on ancestry composition. Given that neither bone appeared to provide a better taxonomic identification over the other, there was insufficient information to support or refute the null hypothesis.

Overall, the results indicated that the skull and os coxa of the *M. mulatta* sample were too morphologically similar for a quantitative approach like GM to allocate individuals taxonomically. Following the research on purebred macaque species (e.g., Kenyon-Flatt, 2020;

Kenyon-Flatt et al., 2020) that has examined morphological variation with a GM approach, it was deemed necessary to determine whether sex had a confounding effect of the sample. Significant values were obtained from a Procrustes ANOVA for each bone, indicating that sex was a confounding variable that needed to be controlled for in the remainder of the statistical analyses. A second set of Procrustes ANOVA examined the ancestry compositions in the sample to analyze whether admixture was causing significant variation in the skull or os coxa. Each bone reported non-significant values, with the skull reporting a *p*-value of 0.9950 and the os coxa reporting 0.0680. These values indicated that the ancestry compositions among individuals did not have a confounding effect on size. Results from the multivariate regression provided further support for the effects of size on the sample, given that size only accounted for roughly 10% of the variation in the skulls and roughly 3% in the pelves. This was an expected outcome given that all individuals included in the sample were adults and were closely related. It is possible that this was simply reporting differences between males and females that were caused by factors unrelated to sexual dimorphism. Providing that the remainder of the variation in each bone was accounted for by shape, additional analysis was necessary to develop a better representation of how hybridization may be affecting morphology.

After performing a PCA on each bone, it was difficult to visualize what was shown after plotting the results because there was a large amount of clustering among individuals and groups. As stated in the results section, the PCA plot could be used to track changes in morphological variation of each bone via the wireframe model along the axes. Variation in the skull dataset was tracked in the overall shape and length of the skull, in addition to along the superior midline and around the zygo-temporal area. In the os coxa dataset, variation was

tracked at the pubic symphysis and iliac crest. Regardless of whether the PCA was examining individuals based on sex or ancestry compositions, there was no clear separation plotted for either bone. Interestingly, however, the hybrid individuals showed greater overall variation in cranial morphology than did either the purebred Chinese or Indian specimens, who all plotted within a subset of the hybrid variation. Conversely, the CVA plots were able to provide a better visualization among the different groups of *M. mulatta* in comparison to the results plotted by the PCA. The CVA plot of the skull dataset was able to distinguish between the purebred Chinese, mostly Chinese, and half hybrid groups, whereas the purebred Indian and mostly Indian groups were clustered together. The CVA plots of the os coxa dataset distinguished between both purebred groups and then clustered all three hybrid groups together. Shape differences were tracked from the wireframe model of the skull at the posterior end, maxilla, and area surrounding the occipital orbit. In the os coxa dataset, the shape differences were tracked at the iliac crest, pubic symphysis, obturator, and ischial tuberosity.

When considering the overall results from the PCA and CVA, there were some patterns that each statistical test registered on the skull and os coxa. The wireframe models examined with the PCA and CVA plots tracked differences in morphological variation in similar anatomical regions on each bone. These similarities may indicate that the overlapping anatomical areas on the skull and os coxa may possess stronger morphological signals. This finding is not unexpected since the PCA and CVA tests were both assessing the morphological variation in the sample, although on different levels (e.g., individual versus group). The difficulty in interpreting the results plotted in the PCA could be explained by how the individuals within the sample all belong to the same species. Given how closely related each population of *M. mulatta* is to one

another, it is not surprising that there was minimal separation in the PCA plot. In addition, all individuals from the sample were housed at the same location and lived as one colony. Therefore, there could be environmental pressures that are causing the lack of morphological variation observed in the sample, which would be affecting both purebred and hybrid individuals. Furthermore, the small number of generations that have passed since the purebred Chinese rhesus macaques were introduced to the Indian rhesus population may have also had an impact on the low level of morphological variation. More specifically, there may have not been enough time that has elapsed for the hybrid individuals to begin displaying significant morphological differences from the purebred populations.

As stated previously, the CVA plots provided better visualization of how the morphological variation differs among groups of ancestry compositions represented in the sample. The CVA uses *a priori* group assignments and investigates how they are related to one another. The name of each ancestry composition was given, but the relationships among each group were unknown by the statistical test. It was interesting to see that the CVA plots clustered the purebred Indian and mostly Indian groups and clearly separated the other three groups based on the morphological variation observed in the skull dataset. Here, it appears that the individuals with more Indian ancestry were more morphologically similar than those with Chinese ancestry. This could indicate that as the amount of Chinese ancestry increases in an individual, the morphology of the skull becomes more differentiated. The CVA plot of the os coxa dataset depicted a different scenario, in that all three hybrid groups were clustered together with each purebred group clearly differentiated. This may indicate that after interbreeding occurs, there are selective pressures on the morphology of the os coxa that

causes it to differentiate from the overall shape that is seen in the purebred populations. Taken together, the results observed from the CVA tests appear to indicate that there may be different morphological signatures in each bone that could be consequences of hybridization that further drive the variation seen among ancestry compositions.

The results from the DFA cross-validation tests suggested that the morphological variation in each bone provided an unrealistic assessment of taxonomic identification. The DFA reported an overall higher accuracy in classifying sex than classifying the ancestry compositions of the sample. The ancestral compositions that reported the highest trends from the cross-validation tests were the mostly Chinese and mostly Indian hybrids. When these groups were subjected to a cross-validation classification with another group from the sample, accuracy was on average higher. The purebred Chinese, purebred Indian, and half hybrids all reported lower average accuracy in cross-validation rates. These trends were similarly reported from the groups regardless of whether the skull or os coxa dataset was investigated. Given that there was a high rate of misclassification among all groups, albeit slightly lower with the mostly Indian and mostly Chinese groups, it is likely that neither skeletal element possessed strong signals of hybridization. That is not to say that there are no morphological signals representing admixture in the *M. mulatta* sample, however, the DFA was unable to register them to make classifications with accuracy.

Overall, the statistical analyses indicated that the purebred and hybrid specimens from the *M. mulatta* sample were able to be distinguished better by sex than by ancestry composition. This not unexpected given that sex was shown to have a confounding effect on the sample. The PCA and DFA tests were unable to distinguish between the morphological

variation among individuals or groups, which caused them to be regularly confused with one another. The CVA test was the only statistical test conducted that reported differences in morphological variation, although there was not enough variation to clearly separate all five groups from each other. To reiterate, there was clear evidence that states **Ho1** and **Ho2** were supported after conclusion of the project. Unfortunately, given that it was not possible to determine which bone was a better indicator of taxonomy, the third hypothesis was not supported or refuted. Again, that is not to say that hybridization was not exerting selective pressures on the morphological variation in the *M. mulatta* sample. When determining how the results from this thesis inform our understanding of hybridization in the field of paleoanthropology, it is necessary to examine how the results presented here compare to those obtained from previous research.

Results obtained from this thesis did not replicate the overall findings from either of the two studies that utilized a GM approach on various purebred macaque species (see Kenyon-Flatt et al., 2020; Kenyon-Flatt, 2020). The goal of those two projects was to determine if morphological variation among different macaque species was significant enough to confirm their existing taxonomic identifications. As stated before, the first project only used two purebred macaque species and the second utilized eight different purebred macaque species. The conclusions from each study stated that there was support for the idea that the purebred macaque samples could be systematically studied in a way that would result in correct taxonomic identification (Kenyon-Flatt et al., 2020; Kenyon-Flatt, 2020).

The first study by Kenyon-Flatt and colleagues (2020) generated scans of two species of captive purebred macaques, one of which was *M. mulatta*. In terms of the results, the smaller

study only ran a PCA to obtain PCs for the DFA tests, therefore the authors did not obtain PCA plots to investigate. The main two statistical tests utilized were the DFA and CVA. The CVA plot that considered species with sexes combined was able to clearly differentiate between groups for the skull and os coxa. There was no clustering among the purebred macaque species. The authors stated that there were distinct morphological differences that were able to be used to identify patterns among groups, while also separating the outgroup that was used as a control. Results from the DFA tests stated that overall, the two purebred macaque species were able to be correctly classified based on the cross-validation tests for each bone. In instances where specimens were misclassified, all macaques were misclassified as the opposite macaque species except for only one specimen being mistaken for the outgroup for the skull dataset. When considering the os coxa dataset, the rate of a correct classification was higher, however, individuals that were misclassified were labeled as macaque or outgroup species (Kenyon-Flatt et al., 2020).

The larger study by Kenyon-Flatt (2020) increased the sample to include eight wild-caught purebred species of macaques, which retained the same two species from the previous study to include *M. mulatta*. Again, the PCA tests were used to provide PCs for the DFA and other statistical analyses, therefore the main results interpreted that overlapped with this thesis were those obtained from the DFA and CVA tests. The findings from the CVA plots were more complex for the skull and os coxa now that there were more groups added to the project. The CVA plot for the skull dataset was able to clearly differentiate the control group from the other purebred macaques, although there was observed clustering among the macaques. The CVA plot for the os coxa dataset showed similar clustering among the purebred macaque

species, while also separating the control group. The DFA cross-validation tests reported a slightly higher misclassification rate than the previous study that only used less macaque species. Given that the misclassification rate increased slightly, the author stated that there was still enough morphological variation present to produce satisfactory results (Kenyon-Flatt, 2020).

Both studies using purebred macaque species reported similar results, which showed that utilizing a GM approach to examine morphological variation appears to be a reliable indicator of taxonomic identification at the species-level. Regardless of whether the sample represented captive or wild-caught purebred macaques, the results from either study still indicated morphological variation among the studied skeletal elements was able to distinguish individuals and groups. In terms of this thesis, it is important to note the aspects of each study that had overlap, regardless of whether the overall findings were similar or not. It is interesting to note that the CVA plots from all three studies observed changes via wireframe axes in similar anatomical regions of the skull and os coxa. Initially, I had suggested that this variation may be a result of selective pressures caused by hybridization. However, since the two studies that utilized purebred macaque species had similar findings, it appears this is no longer the case. As for the differences observed in the DFA and CVA results, this could be explained by the larger sample size and number of groups for the purebred studies. This would explain why the DFA results had higher cross-validation accuracy than the current thesis in that there were more groups being compared to each another. The clustering observed in the CVA plots could have also been explained by how closely related the purebred species are since they are all members of the same genus.

Taken together, the two studies using purebred macaques produced results that would refute my first and second null hypotheses, indicating that the morphological variation among individuals and groups was distinct enough to infer taxonomy. Furthermore, the authors concluded that the skeletal elements of the postcrania were just as effective, and in some cases more so, at inferring the differences in morphology represented by the purebred macaques. This provides support for my third alternative hypothesis, which suggested that the os coxa would be more useful than the skull at labeling morphological differences in the sample. The results obtained from these two studies are important to consider because of the novelty of this nature of research in the field. Based on the results of this thesis, it appears that a GM approach is able to distinguish specimens at the species-level but not at a more specific level considering subspecies hybrids.

Another study, published while this thesis was in progress, also investigated the effects of hybridization on macaque skeletal morphology. Buck and colleagues (2021) utilized the same sample consisting of hybrid Chinese and Indian rhesus macaques to determine how pelvic morphology is differentiated in closely related nonhuman primate species. The goal of this project was to build on existing research that investigated nonhuman primates as an analog to inform our understanding of the hominin fossil record. Pelvic morphology was selected because of the known interactions between locomotive behaviors and parturition in some hybrid nonhuman primates. As a result, the authors hypothesized that this region of the skeleton may vary depending on the ancestry compositions present in the sample of *M. mulatta*. At the time of publication, the authors noted that this was the first study to investigate hybrid pelvic

morphology. In addition, this was the first time that postcranial morphological shape was directly measured in known hybrid primates (Buck et al., 2021).

Similar to this thesis, CT scans of the pelvis were utilized from purebred and hybrid *M. mulatta,* and landmarks were placed to assess morphological variation. After implementing the same GM technique, the authors found that there was no significance in the variation of any one group in the sample, meaning that each displayed similar levels of morphological variation in the os coxa. As such, the different ancestry compositions in the sample were unable to be distinguished from one another. The authors concluded that the results were surprising given previous research on hybrid nonhuman primates, with particular emphasis on observations of hybrid *M. mulatta* in the wild. On average, observations of these two macaque populations in the wild have shown that Chinese *M. mulatta* males are larger than their Indian counterparts and that tail length is longer in Indian *M. mulatta*. In addition, differences in size caused by sexual dimorphism have been noted to be greater in another captive-bred Chinese and Indian hybrid population. The authors suspected there were external selective pressures that were responsible for these visible differences, possibly as a result from each population's respective ecogeography. Despite the differences in the other captive-bred colony, the results did not reflect significant morphological variation in the os coxa even with the Chinese and Indian *M. mulatta* interbreeding for decades (Buck et al., 2021).

Furthermore, the results suggested that the hybrid macaque sample may be a poor analog for hominins. The main point of comparison that suggested the use of Chinese and Indian *M. mulatta* was that this pairing should be similar to *H. sapiens* and Neanderthals. The authors provided a detailed analysis of how each hybrid sample could be analogous to one

another. Factors that were shared among the *M. mulatta* species and the hominin species were 1) a smaller population of closely related species were introduced to the dominant species as seen in the Chinese *M. mulatta* and Neanderthals, 2) the amount of genetic input from the smaller population was estimated to be analogous, and 3) degrees of divergence were argued to have been similar between each taxon pair when introgression occurred (Buck et al., 2021). Overall, Buck and colleagues (2021) were able to show that there is a complex relationship between hybrid morphology and taxonomic divergence, and additional research is required to determine whether results would be replicated in other hybrid nonhuman primates.

Despite the similarities between the current project and that of Buck and colleagues (2021), it was helpful to observe how the results compared given that the same sample of *M. mulatta* was utilized to examine similar research questions. In the context of each study, the morphological variation observed in the macaque skeletal elements was unable to distinguish between individuals in the sample. Both the purebred and hybrid individuals varied minimally in the skull and os coxa, which suggested that hybridization was not strongly affecting the overall morphological shape variation in either bone. The replication of results could be explained by the same sample being used in similar ways to assess skeletal morphology. While this is helpful to show that the results of either study were unlikely erroneous, it would be worth determining whether the same findings are produced from another hybrid nonhuman primate sample. Another interesting outcome from the results that should be noted is that the statistical analyses implemented by Buck and colleagues (2021) found shape differences in similar anatomical areas of the os coxa wireframe axes as the other studies. Given that this new study also tracked shape variation in the same areas of the os coxa, there is further evidence that

these landmarks are indicative of normal morphological variation in *M. mulatta*. In opposition to the results presented by Kenyon Flatt et al. (2020) and Kenyon-Flatt (2020), the projects utilizing hybrid macaque specimens provided support for the notion this GM approach may be unable to differentiate specimens morphologically when assessing specimens below the species-level.

There are several takeaway points from this thesis after comparing the results with the aforementioned research. Two closely related nonhuman primate populations, and their hybrid offspring, were investigated to determine whether the existing morphological variation would be able to infer taxonomic classification. Results indicated that the individuals were unable to be differentiated morphologically, despite being genetically different populations of *M. mulatta*. All individuals included in the sample were housed at the same location and lived in one colony. This raises the question of how the results would track if the study was conducted on another closely related nonhuman primate species that shared environmental pressures. Given that the current study was seeking to find an analogous nonhuman primate species to aid in the identification and classification of hominins, it would be interesting to know if the results would be replicated in a hominin sample. As stated previously, there were periods of time where *H. sapiens* and Neanderthals coexisted and produced fertile hybrid offspring. They too shared a similar environment while hybridization was occurring between species, so what would the results look like in that scenario? Additional research is required before this question can be answered.

Another interesting aspect to consider from the current study was why the statistical analyses were unable to differentiate between *M. mulatta* in the sample. There was ample

speculation provided for why the results were expected or unexpected based on what was seen in other studies, but what if an issue arose elsewhere? Was the sample to blame or the methods? Given that Buck et al. (2021) had similar findings, it is unlikely to be an issue with the sample or with the quantitative method. As it stands, the individuals from the sample were not different enough morphologically to be distinguished by the landmarking protocol of this GM technique. Until further research is conducted, it is unclear whether this type of analysis would ultimately help inform our understanding of the hominin fossil record. As stated in the introduction, hominin fossil identification and classification are already contentious in the field of paleoanthropology. If this GM technique was unable to distinguish between closely related purebred and hybrid macaques, it is possible that it would also be unsuccessful with hominins. An argument can be made that GM methods and the results obtained from the macaque sample would be useful because the genus *Macaca* was identified as the best analog for hominins. Regardless of whether the results may work in a hominin sample, it still updates our understanding of how hybridization is presented in the fossil record. Conversely, an argument can also be made for GM not being a good fit for assessing hominin morphological variation. The current sample of *M. mulatta* was captive-bred and has been since hybridization began introducing hybrids into the colony. Clearly, any hominin species that would be subjected to GM methods would be "wild", therefore the results could end up being completely different. There would need to be further research on how GM methods would assess hybridization in morphological variation of wild nonhuman primate species before this could be tested. Either way, the results would still be informative when addressing questions of morphology throughout evolution.

Lastly, it is important to address any challenges or limitations that arose throughout the duration of the current project. Going into the project, the sample breakdown was unknown in terms of ancestry compositions, and it was unclear how the distribution of ancestry compositions would exist among the *M. mulatta*. The sample was unbalanced and mainly consisted of hybrid individuals (n=109) with very few purebred macaques (n=9). As seen in the results, the sample mainly consisted of mostly Indian hybrid individuals (n=90), which could have affected the PCAs and CVAs. Additionally, the DFA results may have been influenced by the large quantity of hybrid individuals. The mostly Indian hybrid group reported the highest correct classifications in the sample, which could have been caused by there being ninety individuals in this group and only nine purebred macaques. Another aspect of the sample that could have limited the study was the fact that the *M. mulatta* were all captive-bred. As stated before, there is the possibility that the results would have been different if the sample consisted of wild individuals, although it should be noted that it would be much more difficult to obtain ancestry compositions in that scenario.

Another limitation could have been with the scope of the current project. An attempt was made to differentiate individuals and groups based on morphology alone, which did not work with the sample. Clearly there are multiple factors that are affecting the morphology of the sample, and there are many possible selective pressures that could be considered when measuring a hybrid primate species. For the current study, results confirmed the complexity of the interaction between hybridization and morphology, although the goal was primarily to determine how distinct the morphology of the skull and os coxa were in hybrid *M. mulatta*. Further research would be required to examine how hybridization is affecting the morphology

of this sample and considering the interaction of other factors or pressures would be beneficial to see if results were replicated.

This area of research is in its infancy, and clearly there is a need for future research that examines the interaction between hybridization and morphology as it relates to nonhuman primates and hominins. As outlined previously, there are a few directions this research could take. To reiterate, results from the current study indicated that the unbalanced sample could have caused the inability of the GM technique to allocate individuals or groups clearly based on their morphology. If the study could be repeated, it would be beneficial to use a well-balanced sample with similarly sized groups. The other obvious choice when expanding the scope of the current study would be to utilize other nonhuman primate species. Macaques were selected because of their analogous traits with hominins that may have caused their morphology to evolve in a similar manner. Despite the outcome of the thesis, the results do not necessarily state that macaques are a poor analog when addressing the hominin fossil record via GM methods. Given that there are so many species in the genus *Macaca*, it would be worth examining other purebred and hybrid species in *Macaca* and see how the results compare to this *M. mulatta* sample. Depending on the outcomes of those future projects, this would provide further evidence on whether macaques are the best model in this context. In addition, other examples of hybrid nonhuman primates could be subjected to the methods outlined in this thesis to further compare how hybridization may affect their morphology. The use of different nonhuman primate samples could also determine whether there is a better model to use as a comparison for hominin species. Regardless of how future research examines hybrid

morphology, results would update our current understanding of how morphological variation is presented in extinct or extant nonhuman primates, and ultimately hominins.

In conclusion, the current study aimed to provide a better understanding of the hominin fossil record by using an analogous nonhuman primate species. The project attempted to determine how the existing morphological variation of the skull and os coxa in a hybrid *M. mulatta* sample would be characterized when analyzed by a geometric morphometric approach. This would have helped inform how the hybrid morphology of an extant species may have compared to that observed in other primate species, nonhuman and hominin alike. The goals of the current project were unable to be met because the GM methods were unable to clearly distinguish between individuals or groups in the sample based on their morphology alone. The results indicated that the sample was too morphologically similar to differentiate the different populations of *M. mulatta*. Overall, results differed from previous research that has utilized purebred macaque species to measure levels of morphological variation. Alternatively, the results did provide support for another newly published study that used the same sample of *M. mulatta*, which also found that variation was unable to label individuals based on pelvic morphology. It is unclear how the results would differ with another closely related species, like hominins, therefore further research is necessary to elucidate how morphology is affected by hybridization.

Clearly the interaction between hybridization and morphological variation is complex, therefore this is an area of research that needs to be investigated more thoroughly. The initial results provided by research using purebred and hybrid macaques indicates that this GM approach may only be able to differentiate morphological variation at a certain point after the

speciation process occurs. Given that purebred macaques were differentiated, and the hybrid populations were not, this suggests that the morphological consequences of hybridity are at least sometimes undetectable by GM. This finding is important for current issues in the field of paleoanthropology because it states that hybridization and its consequences on skeletal morphology may require more specific techniques to adequately detect or measure. In the context of primate evolution, whether hominin or nonhuman primate, hybridization has clearly impacted the diversity and trajectory of evolution. Addressing the thresholds between species, subspecies, or even hybrid subspecies populations as it relates to morphology has important implications for evolution because it can ensure that our current identification and classification techniques are as accurate as possible. The results presented here address the importance of continuously updating the approaches used in the field, and the research seeking to comparatively analyze primate morphology has only just begun to assess the complexity of hybridization. This nature of research shows considerable promise because any attempt at updating how organisms are classified and defined will ultimately update our understanding of evolutionary history.

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